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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	77.26	77.47

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FILE 'ADISCTI, ADISINSIGHT, ADISNEWS, BIOSIS, BIOTECHNO, CANCERLIT, CAPLUS, CEN, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, DRUGNL, DRUGU, EMBAL, EMBASE, ESBIOBASE, IFIPAT, IPA, JICST-EPLUS, KOSMET, LIFESCI, MEDICONF, MEDLINE, NAPRALERT, NLDB, NUTRACEUT, ...' ENTERED AT 14:59:50 ON 01 JUL 2003

L1 1454 S VITAMIN D AND LUNG AND CANCER
L2 42 S L1 AND VITAMIN D.SUB.2
L3 12 S L1 AND VITAMIN D.SUB.4
L4 12 S L2 AND L3

=> d l4 1-12 bib, kwic

L4 ANSWER 1 OF 12 USPATFULL
AN 2003:81729 USPATFULL
TI Method of treating prostatic diseases using active **vitamin D** analogues
IN Bishop, Charles W., Madison, WI, United States
Knutson, Joyce C., Madison, WI, United States
Mazess, Richard B., Madison, WI, United States
PA Bone Care International, Inc., Middleton, WI, United States (U.S. corporation)
PI US 6537982 B1 20030325
AI US 1998-596149 19980223 (9)
RLI Division of Ser. No. US 1996-781910, filed on 30 Dec 1996, now patented, Pat. No. US 5763429 Continuation-in-part of Ser. No. US 1995-486387, filed on 7 Jun 1995, now patented, Pat. No. US 5798345 Continuation-in-part of Ser. No. US 1995-415488, filed on 3 Apr 1995, now patented, Pat. No. US 5602116 Continuation-in-part of Ser. No. US 1994-265438, filed on 24 Jun 1994, now patented, Pat. No. US 6025346 Continuation-in-part of Ser. No. US 1993-119895, filed on 10 Sep 1993,

'now patented, Pat. No. US 5403831
DT Utility
FS GRANTED
EXNAM Primary Examiner: Criares, Theodore J.
LREP Michael Best & Friedrich LLP, Welch, Teresa J.
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 0 Drawing Figure(s); 0 Drawing Page(s)
LN.CNT 901
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Method of treating prostatic diseases using active **vitamin D** analogues
AB . . . invention provides therapeutic methods for inhibiting, ameliorating or alleviating the hyperproliferative cellular activity of diseases of the prostate, e.g., prostatic **cancer** and prostatic hyperplasia, which includes administering to a patient in need thereof an active **vitamin D** analogue. Cell differentiation is promoted, induced or enhanced without causing to the patient dose-limiting hypercalcemia and hypercalciuria.
SUMM . . . relates generally to a method of treating hyperproliferative prostatic diseases, and in particular, to the use of active forms of **vitamin D** to inhibit the hyperproliferative cellular activity of these diseases and to promote differentiation of the cells.
SUMM . . . prostate gland gives rise to benign prostatic hyperplasia which is one common prostate disease. Another common prostate disease is prostate **cancer**, especially prostatic adenocarcinoma. Adenocarcinoma of the prostate is the most common of the fatal pathophysiological prostate cancers, and typically involves a malignant transformation of epithelial cells in the peripheral region of the prostate gland. Both prostatic hyperplasia and prostate **cancer** have a high rate of incidence in the aging human male population. Approximately one out of every four males above. . .
SUMM Prostate **cancer** is currently the second most frequent cause of **cancer** death after **lung cancer** among American males. Mortality rates for prostate **cancer** increase logarithmically with age and are two times higher in U.S. blacks than whites. Internationally, mortality rates are highest in. . . increase in annual incidence of the disease and a 37% increase in annual mortality rates will be observed. Although prostate **cancer** may be a relatively indolent neoplasm in the elderly, the overall decrease in life span in patients with this disease. . .
SUMM Improvement in the treatment of prostate **cancer** has centered on early detection. In recent years, screening tests which detect certain proteins or peptides secreted by the prostate. . .
SUMM Treatment of prostate **cancer** in men under the age of 65 has focused on radical surgery, e.g., prostatectomy, and/or radiotherapy, but the impact of. . .
SUMM . . . and lumbar vertebrae, causing bone loss and associated pain. Hormone manipulation often may result in significant palliation of metastatic prostate **cancer**, with improvement of bone pain and other disease-associated symptoms. Androgen ablation is, thus, also a major adjunctive therapy in advanced metastatic prostate **cancer** .
SUMM . . . unresectable or metastatic disease will eventually fail to respond to further hormonal therapies. A recent study suggests that human prostate **cancer** cells may cycle between being androgen-independent and androgen-dependent. Such cycling may account for the return of the **cancer** after initial improvement. In this large group of patients, other forms of treatment, unfortunately, are far less effective. Radiotherapy often. . .
SUMM In another area of physiology and biochemistry, the **vitamin D** area, extensive research during the past two decades has

'established important biologic roles for **vitamin D** apart from its classic role in bone and mineral metabolism. Specific nuclear receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3, the hormonally active form of **vitamin D**, are present in cells from diverse organs not involved in calcium homeostasis. For example, Miller et al., 52 **Cancer** Res. (1992) 515-520, have demonstrated specific, biologically active receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3 in the human prostatic carcinoma cell line, LNCaP.

SUMM It has been reported that certain **vitamin D** compounds and analogues are potent inhibitors of malignant cell proliferation and are inducers/stimulators of cell differentiation. For example, U.S. Pat.. . . nonmalignant macrophages (monocytes), and are useful in the treatment of leukemia. Antiproliferative and differentiating actions of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues have been reported with respect to prostate **cancer** cell lines. More recently, an association between **vitamin D** receptor gene polymorphism and prostate **cancer** risk has been reported, suggesting that **vitamin D** receptors may have a role in the development, and possible treatment, of prostate **cancer** .

SUMM These previous studies have focused exclusively on **vitamin D**.sub.3 compounds. Even though these compounds may indeed be highly effective in promoting differentiation in malignant cells in culture, their practical. . . blood calcium levels by virtue of their inherent calcemic activity. That is, the clinical use of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues as anticancer agents is precluded, or severely limited, by the risk of hypercalcemia. This indicates a need for compounds with greater specific activity and selectivity of action, i.e., **vitamin D** compounds with antiproliferative and differentiating effects but which have less calcemic activity. The need for such compounds is no greater. . .

SUMM . . . method of treating prostatic disease conditions such as those characterized by hyperproliferative cell growth and/or abnormal cell differentiation, e.g., prostate **cancer** and prostatic hyperplasia. The method includes use of active **vitamin D** compounds to inhibit abnormal cell growth and promote cell differentiation.

SUMM The 1.alpha.-hydroxyvitamin D compound is an active **vitamin D** and is suitably represented by the formula (I) described hereinafter. Preferred among the compounds of formula (I), are 1.alpha.,24-dihydroxyvitamin D.sub.2,. . .

SUMM In another aspect, the invention is a method of treating human prostate **cancer**, comprising administering to a male subject who has prostate **cancer** an effective amount of an active **vitamin D** compound which has, or attains through metabolism in vivo, a **vitamin D** receptor (VDR) binding affinity substantially equivalent to the binding affinity of 1.alpha.,25-dihydroxyvitamin D.sub.3 and a hypercalcemia risk substantially lower than that of 1.alpha.,25-dihydroxyvitamin D.sub.3, to decrease or stabilize the cellular abnormal proliferative activity of the **cancer**.

SUMM For treatment for prostate conditions in accordance with the present invention, the active **vitamin D** is suitably administered alone as an active ingredient, i.e., as a first anticancer agent, in a pharmaceutical composition, or in. . .

SUMM In another aspect, the invention is a pharmaceutical composition which includes a first anticancer agent which is an active **vitamin D** compound; an agent selected from the group consisting of (i) a second anticancer agent, (ii) a bone agent, (iii) an. . .

DETD . . . relates to therapeutic methods for inhibiting, ameliorating or alleviating the hyperproliferative cellular activity of diseases of the prostate, e.g., prostatic **cancer** and prostatic hyperplasia, and inducing, enhancing or promoting cell differentiation in the diseased cells. The present invention provides a novel treatment of a patient suffering from a hyperproliferative disease such as prostatic **cancer** or prostatic hyperplasia with an active **vitamin D** analogue having a hydrocarbon moiety substituted at the C-24 position of the sidechain of the molecule. Preferably, the active **vitamin D** analogue is a 1.alpha.-hydroxyvitamin D compound and is suitably represented by formula (I) as described hereinbelow. The active **vitamin D** analogue is provided to the patient without causing dose-limiting hypercalcemia and hypercalciuria, i.e., unphysiologically high and deleterious blood calcium levels. . .

DETD . . . accordance with the present invention, when effective amounts of the analogues of formula (I) are administered to patients with prostatic **cancer** or prostatic hyperplasia, the proliferative activity of the abnormal prostatic cells is inhibited or alleviated, and cell differentiation is induced, promoted or enhanced, with significantly less hypercalcemia and hypercalciuria than is observed after the same amount of activated **vitamin D**.sub.3 is administered in previously known formulations. Thus, the compounds of formula (I) have an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues.

DETD It is known that **vitamin D**.sub.3 must be hydroxylated in the C-1 and C-25 positions before it is activated, i.e., before it will produce a biological response. A similar metabolism appears to be required to activate other forms of **vitamin D**, e.g., **vitamin D.sub.2** and **vitamin D.sub.4**. Therefore, as used herein, the term "activated **vitamin D**" or "active **vitamin D**" is intended to refer to a **vitamin D** compound or analogue that has been hydroxylated in at least the C-1 position of the A ring of the molecule. . . and either the compound itself or its metabolites in the case of a prodrug, such as 1.alpha.-hydroxyvitamin D.sub.2, binds the **vitamin D** receptor (VDR). **Vitamin D** compounds which are hydroxylated only in the C-1 position are referred to herein as "prodrugs." Such compounds undergo further hydroxylation. . .

DETD The compound in accordance with the present invention is an active **vitamin D** compound provided that such compound has a hydrocarbon moiety at the C-24 position, e.g., a lower alkyl, alkenyl or acyl group at the C-24 position. Further, the active **vitamin D** in accordance with the present invention may have an unsaturated sidechain, e.g., there is suitably a double bond between C-22. . .

DETD . . . antiproliferative and cell differentiation activity (i.e., reversal of malignant transformation), particularly with respect to cells of prostatic diseases, e.g., prostatic **cancer** and prostatic hyperplasia, but have a lower tendency or inability to cause the undesired side effects of hypercalcemia and/or hypercalciuria.. . . or hyperplastic cell differentiation. The 1.alpha.-hydroxyvitamin D compounds of the present invention, thus, overcome the shortcomings of the known active **vitamin D**.sub.3 compounds described above, and can be considered preferred agents for the control and treatment of malignant diseases such as prostate **cancer** as well as benign prostatic hyperplasia.

DETD Preferred among the active **vitamin D** compounds of formula (I) are: 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin

D.sub.2, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.-hydroxyvitamin D.sub.2, and 1.alpha.-hydroxyvitamin D.sub.4. Among those. . .

DETD The compounds of formula (I) are valuable for the treatment of prostate **cancer** and prostatic hyperplasia in a patient suffering therefrom. In particular, the invention is a method for treating a patient suffering from the hyperproliferative cellular effects of prostate **cancer** and prostatic hyperplasia by administering to the patient a therapeutically effective amount of a compound of formula (I), which is. . .

DETD . . . the compounds of formula (I) have been studied and compared to that of 1.alpha.,25-dihydroxyvitamin D.sub.3, the active hormonal form of **vitamin D** and the standard against which all **vitamin D** compounds and analogues are measured. For example, it has been found that the **vitamin D** receptor (VDR) binding affinities of the compounds of formula (I), or their active metabolites, are substantially equivalent to (i.e., equal. . .

DETD At the same time, it has been found that compounds of formula (I) are significantly less toxic than their corresponding **vitamin D**.sub.3 analogues. For example, in parent co-pending application, Ser. No. 08/265,438, the disclosure of which is incorporated herein by reference, the. . .

DETD . . . in pharmaceutical compositions having reduced side effects and low toxicity as compared with the known analogues of active forms of **vitamin D**.sub.3.

DETD . . . of the pharmaceutical compositions of the present invention is preferred. The dosage of the compounds for the treatment of prostatic **cancer** or hyperplasia according to this invention generally is about 0.01 to about 2.0 .mu.g/kg/day, preferably about 0.01 to about 1.0. . .

DETD For treatment of prostate **cancer**, the parenteral dosage of the compounds of formula (I) is about 0.01 .mu.g/kg/day to about 1.0 .mu.g/kg/day.

DETD Further, included within the scope of the present invention is the co-administration of the active **vitamin D** of formula (I) with a second anticancer agent, e.g., a cytotoxic agent, particularly in metastatic prostate **cancer** wherein relapse has occurred following hormonal treatment. Such agents may suitably include estramustine phosphate, prednimustine, cisplatin, 5-fluoro-uracil, melphalan, hydroxyurea, mitomycin, idarubicin, methotrexate, adriamycin and daunomycin. It is anticipated that an active **vitamin D** of formula (I) used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on. . .

DETD . . . of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. As noted above, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

DETD The affinity of 1.alpha.,24-(OH).sub.2D.sub.2 for the mammalian **vitamin D** receptor (VDR) was assessed using a commercially available kit of bovine thymus VDR and standard 1,25-(OH).sub.2D.sub.3 solutions from Incstar (Stillwater,. . .

DETD The VDR affinity binding of 1.alpha.,24-(OH).sub.2D.sub.4 was investigated. The 1.alpha.,24-(OH).sub.2D.sub.4 was incubated with **vitamin D** receptor and radiolabeled tracer 1.alpha.,25-(OH).sub.2D.sub.3. After incubation, the amount of radioactivity bound to the receptor was determined and compared with. . .

DETD These results show that 1.alpha.,24-(OH).sub.2D.sub.4 binds slightly less tightly to the **vitamin D** receptor than does 1.alpha.,25-(OH).sub.2D.sub.3. Such data mean that 1.alpha.,24-

'(OH).sub.2D.sub.4 has high affinity for the VDR and significant biological activity, similar. . .

DETD . . . results are surprising and unexpected in view of the prior art. They are contrary to the normative wisdom in the **vitamin D** art regarding the very low degree of biological activity of **vitamin D.sub.4** compounds.

DETD VDR binding of **vitamin D** compounds by prostate cells is demonstrated using the techniques of Skowronski et al., 136 Endocrinology (1995) 20-26, which is incorporated. . .

DETD The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,24-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. .

.

DETD The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,25-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. .

.

DETD Using the plasmids p(CT4).sup.4TKGH, a **vitamin D** receptor (VDR)-expressing plasmid, and pSG5-hVDR1/3, a plasmid containing a Growth Hormone (GH) gene, under the control of a **vitamin D**-responsive element (VDRE), experiments were conducted to explore the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to induce **vitamin D**-dependent growth hormone acting as a reporter gene compared to that of 1.alpha.,25-(OH).sub.2D.sub.3. Cells in culture were transfected with these two plasmids. One plasmid contained the gene for Growth Hormone (GH) under the control of the **vitamin D** responsive element (VDRE) and the other plasmid contained the structural gene for the **vitamin D** receptor (VDR). These transfected cultures were incubated with 1.alpha.,24-(OH).sub.2D.sub.4 or 1.alpha.,25-(OH).sub.2D.sub.3, and the production of growth hormone was measured. Table. . .

DETD

TABLE 2

Induction of Growth Hormone by **Vitamin D** Compounds

Compound Used	Concentration (M)	Growth Hormone Induction (ng/ml)
1,25-(OH).sub.2D.sub.3	1 .times. 10.sup.-10	39
1,25-(OH).sub.2D.sub.3	5 .times. 10.sup.-10	248

. . .

DETD These data show that the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to stimulate **vitamin D**-dependent growth hormone is nearly equivalent to that of 1.alpha.,25-(OH).sub.2D.sub.3. Such results are truly surprising and would not have been expected. . .

DETD . . . was conducted to compare the biological activity in vitro of chemically synthesized 1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2, with 1.alpha.,25-(OH).sub.2D.sub.3 and 25-OH-D.sub.3. The **vitamin D**-dependent transcriptional activation model system was used in which plasmids pSG5-hVDR1/3 and p(CT4).sup.4TKGH were co-transfected into Green monkey kidney, COS-1 cells.

DETD Transfected cells were incubated with **vitamin D** metabolites and growth hormone production was measured. As shown in Table 3, both 1.alpha.,24(S)-(OH).sub.2D.sub.2 and its epimer, 1.alpha.,24(R)-(OH).sub.2D.sub.2, had significantly. . .

DETD

TABLE 3

Vitamin D-Inducible Growth Hormone Production

Ih Transfected COS-1 Cells
Vitamin D-Inducible Growth
Hormone Production
Net
Total GH **vitamin D**-inducible
Molar Production* GH-production
Inducer Concentration (ng/ml) (ng/ml)

Ethanol 44 0
25-OH-D.sub.3 1 .times. 10.sup.-7 245 201
1 .times. 10.sup.-6 1100 1056
1 .times.. . .

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. Medium containing test analogue or vehicle is replaced every three. . .

DETD The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue, 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. After 6-7 days, the medium is removed and stored at. . .

DETD The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,24-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,25-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD Patients with advanced androgen-independent prostate **cancer** participate in an open-labeled study of 1.alpha.,24-(OH).sub.2D.sub.2. Qualified patients are at least 40 years old, exhibit histologic evidence of adenocarcinoma. . . patients begin a course of therapy with oral 1.alpha.,24-(OH).sub.2D.sub.2 lasting 26 weeks, while discontinuing any previous use of calcium supplements, **vitamin D** supplements, and **vitamin D** hormone replacement therapies. During treatment, the patients are monitored at regular intervals for: (1) hypercalcemia, hyperphosphatemia, hypercalciuria, hyperphosphaturia and other. . .

DETD The study of Example 14 is repeated for the active **vitamin D** compound, 1.alpha.-OH-D.sub.2. The results of the phase one study indicate that patients treated with the MTD of 1.alpha.-OH-D.sub.2 for at. . .

CLM What is claimed is:
1. A pharmaceutical combination comprising a first anticancer agent which is an active **vitamin D** compound; and (b) a second anticancer agent, wherein the active **vitamin D** compound is a 1.alpha.-hydroxyvitamin D compound having a hydrocarbon moiety substituted at C-24.

2. A pharmaceutical combination comprising a first anticancer agent

which is an active **vitamin D** compound; and (b) a second anticancer agent, wherein the active **vitamin D** compound is 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin D.sub.2, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.-hydroxyvitamin D.sub.2 or 1.alpha.-hydroxyvitamin D.sub.4.

L4 ANSWER 2 OF 12 USPATFULL
AN 2002:236042 USPATFULL
TI Treatment of hyperproliferative diseases using active **vitamin D** analogues
IN Mazess, Richard B., Madison, WI, UNITED STATES
PA Bone Care International, Inc., Middleton, WI (U.S. corporation)
PI US 2002128240 A1 20020912
AI US 2001-995911 A1 20011128 (9)
RLI Continuation-in-part of Ser. No. US 2001-891814, filed on 26 Jun 2001, PENDING Continuation-in-part of Ser. No. US 1998-596149, filed on 23 Feb 1998, PENDING Division of Ser. No. US 1996-781910, filed on 30 Dec 1996, PATENTED
DT Utility
FS APPLICATION
LREP MICHAEL BEST & FRIEDRICH, LLP, ONE SOUTH PINCKNEY STREET, P O BOX 1806, MADISON, WI, 53701
CLMN Number of Claims: 41
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1385
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Treatment of hyperproliferative diseases using active **vitamin D** analogues
AB . . . present invention provides a method of inhibiting the hyperproliferative cellular activity of neoplasms and other hyperproliferative diseases with an active **vitamin D** compound utilizing a high dose, episodic treatment protocol.
SUMM [0003] This invention relates to a method of treating hyperproliferative diseases utilizing active forms of **vitamin D**. The active **vitamin D** compound inhibits the hyperproliferative cellular activity of these diseases and promotes differentiation of the cells with reduced risk of hypercalcemia. The reduced risk of hypercalcemia is achieved 1) by episodic administration of high dose active **vitamin D**; or 2) by episodic co-administration of the active **vitamin D** with an antihypercalcemic agent such as a bisphosphonate. The risk is further mitigated where the active **vitamin D** compound is a hypocalcemic active **vitamin D**. The present invention also provides a pharmaceutical combination therapy in which the active **vitamin D** compound is co-administered with other antineooplastic (i.e., anticancer) agents. The methods of present invention are also useful in controlling, stabilizing. . .
SUMM [0004] Extensive research during the past two decades has established important biologic roles for **vitamin D** apart from its classic role in bone and mineral metabolism. Specific nuclear receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3, the hormonally active form of **vitamin D**, are present in cells from diverse organs not involved in calcium homeostasis. For example, specific, biologically active **vitamin D** receptors have been demonstrated in the human prostatic carcinoma cell line, LNCaP, (Miller et al., 52 **Cancer** Res. (1992) 515-520); **vitamin D** receptors have also been described for many other neoplastic cells, e.g., carcinomas of the breast and the colon.
SUMM [0005] It has been reported that certain **vitamin D**

compounds and analogues are potent inhibitors of malignant cell proliferation and are inducers/stimulators of cell differentiation. For example, U.S. Pat.. . . nonmalignant macrophages (monocytes), and are useful in the treatment of leukemia. Antiproliferative and differentiating actions of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues have also been reported with respect to **cancer** cell lines. More recently, an association between **vitamin D** receptor gene polymorphism and **cancer** risk has been reported, suggesting that **vitamin D** receptors may have a role in the development, and possible treatment, of **cancer**.

SUMM [0006] Previous studies of **vitamin D** compounds and **cancer** treatment have focused exclusively on **vitamin D**.sub.3 compounds. Even though these compounds may indeed be highly effective in promoting differentiation in malignant cells in culture, their practical. . . blood calcium levels by virtue of their inherent calcemic activity. That is, the clinical use of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues as anticancer agents is precluded, or severely limited, by the risk of hypercalcemia. This indicates a need for compounds with greater specific activity and selectivity of action, i.e., **vitamin D** compounds with antiproliferative and differentiating effects but which have less calcemic activity.

SUMM [0007] In addition to the risk of hypercalcemia associated with clinical use of certain **vitamin D** compounds that are potent stimulators of intestinal calcium absorption, hypercalcemia has now also been specifically associated with malignancy. Such malignancy. . . the 1,25-dihydroxyvitamin D.sub.3 compounds themselves increase serum calcium levels. Accordingly, a need also exists for specific treatment regimens of active **vitamin D** that will provide antiproliferative and differentiating effects yet control PTHrP levels.

SUMM . . . characterized by hyperproliferative cell growth and/or abnormal cell differentiation, with reduced risk of hypercalcemia. The method includes use of active **vitamin D** compounds (defined hereinafter), and of particular value, hypocalcemic active **vitamin D** compounds, especially of vitamins D.sub.2 and D.sub.4, in high dosage form, administered on an intermittent or episodic basis, to inhibit abnormal cell growth and promote cell differentiation. The active **vitamin D** compound may be used as sole therapy or may be used in combination therapy with one or more other antineoplastic agents. An antihypercalcemia agent may also be used with the active **vitamin D** or with the **vitamin D**-antineoplastic agent combination. A high dosage episodic regimen of active **vitamin D** is also of value in controlling serum PTHrP level, the elevation of which is correlated with hypercalcemia associated with malignancies. . .

SUMM . . . inhibiting the hyperproliferative activity of neoplastic or hyperplastic cells, comprising treating the cells with an effective amount of an active **vitamin D** compound. The treating step includes inhibiting proliferation of, and inducing and enhancing differentiation in such cells. The effective amount of the active **vitamin D** is provided by a high dose, episodic administration regimen. The methods of the present invention are also of value in. . .

SUMM [0010] The **vitamin D** compound of the present invention is an active **vitamin D** and is generally represented by the formula (I) described hereafter. The active **vitamin D** compounds of the present invention include **vitamin D** compounds having a hydroxy group substituted in at least one of the C.sub.1, C.sub.24 or C.sub.25 positions of the molecule, i.e., a hydroxy **vitamin D**. For example, compounds of formula (I) suitably include, without limitation,

1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin D.sub.2, 1.alpha.,25-dihydroxyvitamin D.sub.3,. . .

SUMM [0011] The active **vitamin D** compounds in accordance with the present invention are valuable for the treatment of breast and colon or colorectal **cancer**, as well as other neoplasms such as pancreatic **cancer**, prostate **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adenocarcinoma and large cell types), squamous cell **cancer** of the head and neck, bladder, ovarian and cervical cancers, myeloid and lymphocytic leukemia, lymphoma, hepatic tumors, medullary thyroid carcinoma,. . . of the soft tissue and bone. Concomitant with its value in treatment of hyperproliferative diseases, neoplasms and malignancies, the active **vitamin D** compounds in accordance with the present invention are beneficial in lowering and/or maintaining lowered PTHrP levels, the elevation or overproduction. . .

SUMM [0012] In accordance with the present invention, when effective amounts of active **vitamin D** compounds are administered to patients with **cancer** or neoplasms, the proliferative activity of the abnormal neoplastic cells is inhibited, reduced, or stabilized, and/or cell differentiation is induced,. . .

SUMM [0013] The effective amounts of **vitamin D** compound are given in an administration protocol of high dosage, generally 10 .mu.g/dose or greater up to 200 .mu.g/dose or. . . The protocol or dosage regimen in accordance with the present invention provides an improved therapeutic index for active forms of **vitamin D** analogues compared to administration via conventional regimens. The episodic dosing is also cost effective as less active agent is needed.

SUMM [0014] Accordingly, another aspect of the invention is a method of treating human **cancer** comprising administering to a subject who has **cancer** an effective amount of **vitamin D** compound which has, or attains through metabolism in vivo, a **vitamin D** receptor (VDR) binding affinity substantially equivalent to the binding affinity of 1.alpha.,25-dihydroxyvitamin D.sub.3 and a hypercalcemia risk substantially lower than. . . D.sub.3 given in known or conventional treatment regimens, to inhibit, decrease or stabilize the cellular abnormal proliferative activity of the **cancer**. Such hypocalcemic active **vitamin D** compounds further mitigate the risk of hypercalcemia because of their inherent lower calcemic index.

SUMM [0015] For treatment for malignant conditions in accordance with the present invention, the active **vitamin D** compounds of formula (I) can be suitably administered alone as an active ingredient, i.e., as an antiproliferative agent, in a. . . or co-administered as described hereinbelow with other therapeutic agents, e.g., anticancer (i.e., antiproliferative, cytotoxic, antitumor or antineoplastic) agents. The active **vitamin D** compound is given in episodic or intermittent high dose. Administration of the active **vitamin D** may be prior to, simultaneous with, or after administration of the other therapeutic agents.

SUMM [0016] Specifically included within the scope of the present invention is the co-administration of the active **vitamin D** of formula (I) with a cytotoxic or anticancer agent; in other words, a combination therapy or treatment. Cytotoxic or antineoplastic. . .

SUMM [0017] It is anticipated that the **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic or antineoplastic effect on. . .

SUMM . . . of hormones or other therapeutic agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. For example,

prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

SUMM [0019] In another aspect, the invention is a pharmaceutical combination which includes an anticancer agent which is an active **vitamin D** compound and an agent selected from the group consisting of (i) an anticancer (or antineoplastic or antihyperproliferative) agent, (ii) a. . . value as bone agents can also be used to mitigate hypercalcemia. Thus, the co-administration of a bisphosphonate with an active **vitamin D** compound or with an active **vitamin D** compound and a cytotoxic or antineoplastic agent combination therapy is desirable for further mitigating the risk of hypercalcemia.

SUMM [0020] All routes of administration of the active **vitamin D** or its co-administration with other therapeutic agents are suitable. However, parenteral administration of the active **vitamin D** compounds in accordance with the present invention, alone or in combination with other agents, provides advantages over other treatment modalities.. . .

SUMM . . . in the diseased cells. The present invention provides treatment of a patient suffering from a hyperproliferative disease, such as prostatic **cancer** or prostatic hyperplasia, with an active **vitamin D** analogue or compound based on a novel treatment protocol. The active **vitamin D** compound is suitably a hydroxy **vitamin D**, e.g., a 1.alpha.-hydroxyvitamin D, a 24-hydroxyvitamin D or a 25-hydroxyvitamin D compound. The active **vitamin D** analogue represented by formula (I) as described hereinbelow is provided to the patient with significantly reduced risk of or without. . . deleterious blood calcium levels and urine calcium levels, respectively. These attributes are achieved through specific chemical properties of the active **vitamin D** compounds and the novel treatment protocol as described herein.

SUMM [0024] In accordance with the present invention, when effective amounts of the active **vitamin D** compounds are administered to patients with **cancer** or hyperplasia, the proliferative activity of the abnormal cells is inhibited, maintained, or alleviated, and cell differentiation is induced, promoted or enhanced, with significantly less risk of hypercalcemia and hypercalciuria than is observed after the same amount of activated **vitamin D** .sub.3 is administered in previously known formulations and dosing regimens. The risk of hypercalcemia, long associated with the administration of high doses of **vitamin D** compounds, is lowered (1) by administering an active **vitamin D** on an intermittent or episodic basis, especially by administering hypocalcemic active **vitamin D** compound, or (2) by co-administering the active **vitamin D** compound an antihypercalcemic agent on an intermittent or episodic basis. Thus, the active **vitamin D** compounds for use in accordance with the present invention have an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues given in conventional protocols. The treatment protocol in accordance with the present invention provides reduced risk of hypercalcemia, e.g.,. . .

SUMM [0025] It is known that **vitamin D**.sub.3 must be hydroxylated in the C-1 and C-24 or C-25 positions before it is activated, i.e., before it will produce a biological response. A similar metabolism appears to be required to activate other forms of **vitamin D**, e.g., **vitamin D**. **sub.2** and **vitamin D**.**sub**. **4**. Therefore, as used herein, the term "activated **vitamin D**" or "active **vitamin D**" is intended to refer to a **vitamin D** compound or

analogue that has been hydroxylated in at least one of the C-1, C-24 or C-25 positions of the. . . and either the compound itself or its metabolite in the case of a prodrug, such as 1.alpha.-hydroxyvitamin D.sub.2, binds the **vitamin D** receptor (VDR). For example, **vitamin D** "prodrugs" or "prohormones" include compounds which are hydroxylated in only one of the three positions. Such compounds undergo further hydroxylation. . .

SUMM [0028] The compound in accordance with the method of the present invention is an active **vitamin D** compound. The active **vitamin D** in accordance with the present invention may have an unsaturated sidechain, e.g., there is suitably a double bond between C-22. . .

SUMM [0029] An active **vitamin D** of the present invention, i.e., a hydroxyvitamin D, has the general formula described in formula (I) ##STR1##

SUMM . . . a double bond. Compounds of formula I,II and III in which Y is hydrogen are also referred to as 19-nor **vitamin D** compounds.

SUMM . . . fluoroalkyl, lower fluoroalkenyl, O-lower alkyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl or lower cycloalkyl. These are compounds or analogues of **vitamin D.sub.2** and **vitamin D.sub.4**. Of particular value are those **vitamin D.sub.2** and D.sub.4 compounds where X.sup.1, X.sup.2 or X.sup.3 are hydroxyl. Such compounds include 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin. . . 24-hydroxyvitamin D.sub.4; they are typically hypocalcemic compared to the natural D hormone, 1.alpha.,25-dihydroxyvitamin D.sub.3. By "hypocalcemic" is meant an active **vitamin D** compound that has reduced calcemic activity compared to that of the natural **vitamin D** hormone, 1.alpha.,25-dihydroxyvitamin D.sub.3; in other words, a calcemic index less than that of 1.alpha.,25-dihydroxyvitamin D.sub.3. "Calcemic index" is a relative. . . of a drug to generate a calcemic response, the calcemic activity of 1.alpha.,25-dihydroxyvitamin D.sub.3 being designated as 1. Such hypocalcemia **vitamin D** compounds provide reduced risk of hypercalcemia even when administered in high doses.

SUMM . . . hyperproliferative cells, (i.e., inhibiting their hyperproliferative activity and/or inducing and enhancing their differentiation) with an effective amount of an active **vitamin D** compound. The effective dosage amount administered to a patient having a hyperproliferative disease is a high dose of active **vitamin D** compound, including 1.alpha.,25-dihydroxyvitamin D.sub.3 (calcitriol), given on an intermittent or episodic dosing regimen. By "high dose" is meant a dose. . . 200 .mu.g. In other terms, a "high dose" is one that produces in vivo higher than normal physiologic levels of **vitamin D**, or is sufficient in a single dose to upregulate **vitamin D** receptors on cells expressing these receptors. The intermittent dosing regimen is suitably between once per week to once every 12. . .

SUMM [0039] Each single dose is sufficient to upregulate **vitamin D** hormone receptors in target cells. It is believed that continuous dosing is not required because the binding and upregulation by **vitamin D** compounds is sufficient to initiate the cascade of intracellular metabolic processes occurring with receptor binding. Intermittent dosing reduces the risk. . . thus, the method in accordance with the present invention can be used to treat hyperproliferative diseases by administering any active **vitamin D** compound. At the same time, it is contemplated, in accordance with the present invention, that the risk of hypercalcemia can be further mitigated if the active **vitamin D** compound

is a hypocalcemic active **vitamin D** compound.

SUMM . . . The compounds of the present invention given in the illustrated dosing regimen, thus, overcome the shortcomings of the known active **vitamin D**.sub.3 compounds described above, and can be considered preferred agents for the control and treatment of malignant diseases such as breast, prostate, testicular and colon or colorectal **cancer**, as well as other neoplasms such as pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . medullary thyroid carcinoma, multiple myeloma, melanoma, retinoblastoma, and sarcomas of the soft tissue and bone, i.e. neoplasms that express a **vitamin D** receptor. Hyperproliferative conditions that may be treated by the method of the present invention also include psoriasis and hyperplasias such. . .

SUMM . . . believed that the intermittent high dose regimen can be used to effect any therapeutic effect that is attributable to active **vitamin D**., e.g., antiproliferative activity, reduction of loss of bone mass, etc. In regard to antiproliferative activity, the value of the intermittent dosing is that antihyperproliferative activity and upregulation of **vitamin D** receptors occurs with a single dose without the side effects of hypercalcemia and hypercalciuria that occur with recurrent daily dosing.. . .

SUMM . . . total dose is given. The compounds in accordance with the present invention are administered in an amount that raises serum **vitamin D** levels to a supraphysiological level for a sufficient period of time to induce differentiation or regression of a tumor or neoplasm without causing hypercalcemia or with substantially reduced the risk of hypercalcemia. The properties of the hypocalcemic **vitamin D** compounds are particularly beneficial in permitting such supraphysiologic levels.

SUMM . . . conventional methods of pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans. For example, the active **vitamin D** compounds of the present invention can be formulated in pharmaceutical compositions in a conventional manner using one or more conventional. . .

SUMM . . . parenteral, e.g., injectable, dosage form. Using the parenteral route of administration allows for bypass of the first pass of active **vitamin D** compound through the intestine, thus avoiding stimulation of intestinal calcium absorption, and further reduces the risk of esophageal irritation which. . .

SUMM [0049] Although it is considered that episodic parenteral administration of high dose active **vitamin D** is highly beneficial, it is also contemplated within the scope of the present invention that enteral dosing, e.g., oral administration, can also be of benefit. Thus, episodic enteral dosing of high dose active **vitamin D** is also considered of benefit in achieving the upregulation of cell receptors and control of PTHrP in treatment of hyperproliferative. . .

SUMM [0061] The dosage of the compounds for the treatment of **cancer** or neoplasms with the active **vitamin D** compounds in accordance with the present invention can be done on an episodic basis, in which high doses can be. . .

SUMM . . . about 0.01 .mu.g to about 50 .mu.g per gram of composition. For treatment of skin cancers, the dosage of the **vitamin D** compound in a locally applied composition generally is about 0.01 .mu.g to 100 .mu.g per gram composition.

SUMM [0064] Further, included within the scope of the present invention is a method of co-administration of active **vitamin D** compounds with an anticancer or antineoplastic agent. In accordance with the present invention, therapeutic antihyperproliferative benefits are

achieved with intermittent dosing of active **vitamin D** with cytotoxic, i.e., other chemotherapeutic or antineoplastic, agents. Many antineoplastic or cytotoxic agents must be delivered through a parenteral route of administration, and thus, a protocol of injectable active **vitamin D** and antineoplastic agent can be set up on a routine basis. The co-administration of active **vitamin D** and antineoplastic agents can be prior to, after, or simultaneous with each other. However, it is believed that the prior administration of active **vitamin D** with the later episodic administration of a cytotoxic or antineoplastic agent is of benefit. For example, the high dose active **vitamin D** upregulates the receptors, and primes and promotes cell differentiation. Such upregulation and priming, potentially permits less cytotoxic or antineoplastic agent. . .

SUMM . . . other at a later time, e.g., within a week. An example of a suitable co-administration regimen is where an active **vitamin D** compound is administered from 0.5 to 7 days prior to administration of a cytotoxic or antineoplastic agent.

SUMM [0068] It is anticipated that active **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic or antineoplastic effect on. . .

SUMM [0069] Also included within the scope of the present invention is the co-administration of effective dosages of active **vitamin D** compounds with hormones or other agents, e.g., estrogens, that are known to ameliorate bone diseases or disorders. For example, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

SUMM [0072] Combinations of these therapeutic agents, some of which have also been mentioned herein, with an active **vitamin D** compound will bring additional, complementary, and often synergistic properties to enhance the desirable properties of these various therapeutic agents. In such combination therapy, the active **vitamin D** compound may be administered with the other therapeutic agent (e.g., concurrently, concombinantly, sequentially, or in a unitary formulation) such that. . .

DETD [0074] VDR binding of **vitamin D** compounds by prostate cells is demonstrated using the techniques of Skowronski et al., 136 Endocrinology (1995) 20-26, which is incorporated. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.** **4**[1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0075] The procedure of Example 1 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,24-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. . .

DETD [0076] The procedure of Example 1 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,25-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.** **4**[1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0077] Using the plasmids p(CT4).sup.4TKGH, a **vitamin D** receptor (VDR)-expressing plasmid, and pSG5-hVDR1/3, a plasmid containing growth hormone (GH) gene under the control of a **vitamin D**-responsive element (VDRE), experiments were conducted to explore the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to induce **vitamin D**-dependent growth hormone acting as a reporter gene compared to that of 1.alpha.,25-(OH).sub.2D.sub.3. Cells in culture were transfected with these two. . . of growth hormone was

measured. Table 2 below shows the results of this assay:

TABLE 2

Induction of Growth Hormone by **Vitamin D** Compounds

Compound	Concentration Used (M)	Growth Hormone Induction (ng/ml)
1,25-(OH).sub.2D.sub.3	1 .times. 10.sup.-10	39
1,25-(OH).sub.2D.sub.3	5 .times. 10.sup.-10	248
1,24-(OH).sub.2D.sub.4.	. .	

DETD [0078] These data show that the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to stimulate **vitamin D**-dependent growth hormone is nearly equivalent to that of 1.alpha.,25-(OH).sub.2D.sub.3. Such results are truly surprising and would not have been expected. . .

DETD 1.alpha.,24(S)-dihydroxyvitamin D.sub.2 and 1.alpha.,24(R)-dihydroxy-**vitamin D.sub.2**
[1.alpha.,24(S)--(OH).sub.2D.sub.2 and 1.alpha.,24(R)--(OH).sub.2D.sub.2]

DETD . . . was conducted to compare the biological activity in vitro of chemically synthesized 1.alpha.,24(S)--(OH).sub.2D.sub.2 and 1.alpha.,24(R)--(OH).sub.2D.sub.2, with 1.alpha.,25-(OH).sub.2D.sub.3 and 25-OH--D.sub.3. The **vitamin D**-dependent transcriptional activation model system was used in which plasmids pSG5-hVDR1/3 and p(CT4).sup.4TKGH were co-transfected into Green monkey kidney, (COS-1) cells.

DETD [0080] Transfected cells were incubated with **vitamin D** metabolites and growth hormone production was measured. As shown in Table 3, both 1.alpha.,24(S)--(OH).sub.2D.sub.2 and its epimer, 1.alpha.,24(R)--(OH).sub.2D.sub.2, had significantly more activity in this system than 25-OH--D.sub.3, with 1.alpha.,24(S)--(OH).sub.2D.sub.2 having nearly the same activity as 1.alpha.,25-(OH).sub.2D.sub.3.

TABLE 3

Vitamin D-Inducible Growth Hormone Production In Transfected COS-1 Cells

Inducer	Molar Concentration	Vitamin D Inducible Growth Hormone Production Total GH Production* (ng/ml)	Net **vitamin D** inducible GH-production (ng/ml)
Ethanol		44	0
25-OH-D.sub.3	1 .times. 10.sup.-7	245	201
	1 .times. 10.sup.-6	1100	1056
	1. . . .		

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. Medium containing test analogue or vehicle is replaced every three. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.4**[1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0082] The procedure of Example 6 is repeated using the active **vitamin D** compound 1.alpha.,24-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD [0083] The procedure of Example 6 is repeated using the active

vitamin D compound 1.alpha.,25-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue, 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. After 6-7 days, the medium is removed and stored at. . .

DETD [0086] The procedure of Example 9 is repeated except the active **vitamin D** compound is 1.alpha.,24-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD [0087] The procedure of Example 9 is repeated except the active **vitamin D** compound is 1.alpha.,25-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD [0088] Patients with a known **vitamin D** receptor positive tumor (e.g., adenocarcinoma of the prostate, breast, **lung**, colon or pancreas, or transitional cell carcinoma of the bladder, or melanoma) participate in an open-label study of an active **vitamin D** compound in accordance with the present invention. Patients are placed on a reduced calcium diet prior to treatment, to help minimize intestinal absorption and allow ever higher doses of the active **vitamin D**. This reduced calcium diet may be continued for the duration of treatment, and for one week after the last dose of the active **vitamin D**. The diet ideally restricts daily calcium intake to 400-500 mg. Patients also discontinue use of any **vitamin D** supplements or **vitamin D** replacement therapies. Each patient is also asked to drink 4-6 cups of fluid more than usual intake to assure adequate. . .

DETD Treatment of Prostate **Cancer** with 1.alpha.,24-dihydroxy **vitamin D.sub.2** [1 .alpha.,24-(OH).sub.2D.sub.2]

DETD [0092] Patients with advanced androgen-independent prostate **cancer** participate in an open-label study of 1.alpha.,24-(OH).sub.2D.sub.2. Qualified patients are at least 40 years old, exhibit histologic evidence of adenocarcinoma. . . a course of therapy with oral or intravenous 1.alpha.,24-(OH).sub.2D.sub.2 lasting 26 weeks, while discontinuing any previous use of calcium supplements, **vitamin D** supplements, and **vitamin D** hormone replacement therapies. During treatment, the patients are monitored at regular intervals for: (1) hypercalcemia, hyperphosphatemia, hypercalciuria, hyperphosphaturia and other. . .

DETD Treatment of Prostate **Cancer** with 1.alpha.-hydroxyvitamin D.sub.2[1.alpha.-OH--D.sub.2]

DETD [0096] The study of Example 13 is repeated for the active **vitamin D** compound, 1.alpha.-OH--D.sub.2. The results of the phase one study indicate that patients treated with the 20 .mu.g of 1.alpha.-OH--D.sub.2 once. . .

DETD Treatment of Liver **Cancer**

DETD Treatment of **Cancer** by Episodic Co-administration of Active **Vitamin D** and an Antineoplastic Agent

DETD [0100] Patients with malignant tumors participate in a treatment regimen of 1.alpha.,24-(OH).sub.2D.sub.2 and paclitaxel. Both the active **vitamin D** and paclitaxel are given intravenously. Paclitaxel is given in a 3-hour infusion, once every 3 weeks with the active **vitamin D** co-administered once every 3 weeks for 26 weeks. The dosage of paclitaxel is 80 mg/m.sup.2 and the

1.alpha.,24-(OH).sub.2D.sub.2 is 50. . .

DETD Treatment of **Cancer** by Co-administration of Active **Vitamin D** Compound, an Antineoplastic Agent and an Antihypercalcemic Agent

DETD . . . to treat patients with malignant tumors by a treatment regimen that includes an antihypercalcemic agent as well as the active **vitamin D** and the antineoplastic agent. The treatment regimen includes, e.g., 1.alpha.-OH--D.sub.2, 1.alpha.,24-(OH).sub.2D.sub.2 or 1.alpha.,25-(OH).sub.2D.sub.3, paclitaxel and pamidronate. All active agents are. . .

CLM What is claimed is:

. . . of inhibiting hyperproliferation of malignant or neoplastic cells, comprising treating the cells episodically with an antiproliferative amount of an active **vitamin D** compound which is a hypocalcemic **vitamin D** compound having a hydrocarbon moiety at the C.sub.24 position, with reduced risk of hypercalcemia; the cells expressing a **vitamin D** receptor.

2. The method as claimed in claim 1 wherein the active **vitamin D** compound is a hypocalcemic **vitamin D** compound.

3. The method of claim 1, wherein the malignant cells are associated with cancers of the breast, colon, prostate, **lung**, neck and head, pancreas, endometrium, bladder, cervix, testes, ovaries, squamous cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma,. . .

4. The method of claim 2, wherein the hypocalcemic **vitamin D** is a compound represented by formula (I): ##STR4## wherein A.sup.1 and A.sup.2 each are hydrogen or together represent a carbon-carbon. . .

5. A method in accordance with claim 2 wherein the hypocalcemic **vitamin D** compound is a compound of formula (II): ##STR5## wherein A.sup.1 and A.sup.2 each are hydrogen or together represent a carbon-carbon. . .

6. A method in accordance with claim 2, wherein the hypocalcemic **vitamin D** compound is a compound of formula (III): ##STR6## wherein A.sup.1 and A.sup.2 each are hydrogen or together represent a carbon-carbon. . .

7. The method of claim 2 wherein the active **vitamin D** is 1.alpha.-hydroxyvitamin D.sub.2 or 1.alpha.,24-dihydroxyvitamin D.sub.2.

8. The method of claim 2 wherein the active **vitamin D** is 1.alpha.-hydroxyvitamin D.sub.4; 1.alpha.,25-dihydroxyvitamin D.sub.2; 1.alpha.,24,25-trihydroxyvitamin D.sub.2 1.alpha.,25-dihydroxyvitamin D.sub.4; 1.alpha.,24,25-trihydroxyvitamin D.sub.4; 24-hydroxyvitamin D.sub.2; or 24-hydroxyvitamin D.sub.4.

9. The method as claimed in claim 2 wherein an amount of the active **vitamin D** compound is episodically administered to a human **cancer** patient, the amount being effective to inhibit the hyperproliferation of the neoplastic cells with reduced risk of hypercalcemia.

10. The method as claimed in claim 9 wherein the amount of active **vitamin D** is a high dose which is between about 10 .mu.g to about 200.mu.g.

11. The method of claim 9 wherein the amount of the **vitamin D** compound is administered parenterally or orally in combination with a pharaceutically acceptable carrier.

12. The method of claim 11 wherein the amount of **vitamin D** compound is administered parenterally.

13. The method of claim 12 wherein the amount of **vitamin D** compound is administered intravenously.

15. The method of claim 1 wherein the active **vitamin D** lacks a hydrocarbon moiety at the C-24 position.

16. The method of claim 15 wherein the active **vitamin D** is 1.alpha.,25-dihydroxyvitamin D.sub.3 or 1.alpha.-dihydroxyvitamin D.sub.3.

17. The method of claim 16 wherein the amount of the **vitamin D** compound is administered parenterally or orally in combination with a pharmaceutically acceptable carrier.

18. The method of claim 17 wherein the amount of **vitamin D** compound is administered parenterally.

19. The method of claim 18 wherein the amount of **vitamin D** compound is administered intravenously.

. . . of inhibiting hyperproliferation of malignant or neoplastic cells, comprising treating the cells by co-administering an antihyperproliferative amount of an active **vitamin D** compound and an effective amount of an agent which is an antineoplastic agent, a bone agent, an antihypercalcemic agent or combinations thereof, the cells expressing a **vitamin D** receptor, the antiproliferative amount of the active **vitamin D** compound being administered on an episodic basis which is once per week to about once per 12 weeks.

22. The method of claim 21 wherein an amount of the active **vitamin D** compound and an amount of the agent are episodically co-administered to a human **cancer** patient, the amount of the active **vitamin D** effective to inhibit the hyperproliferation of the neoplastic cells.

24. The method of claim 23 wherein the antineoplastic agent is given episodically and the active **vitamin D** is given concurrently with the antineoplastic agent.

28. The method of claim 22 wherein an active **vitamin D** compound, an antineoplastic agent and an antihypercalcemic agent are co-administered.

. . . a hyperproliferative diseasé, comprising treating the cells with an antihyperproliferative amount of an active D compound, the cells expressing a **vitamin D** receptor, the antiproliferative amount of the active **vitamin D** compound being administered on an episodic basis which is once per week to about once per 12 weeks.

30. The method of claim 29 wherein an amount of the active **vitamin D** compound is episodically administered to a human patient suffering from the hyperproliferative disease, the amount being effective to inhibit hyperproliferation. . .

33. A pharmaceutical therapy, comprising episodic co-administration of an active **vitamin D** compound with an antineoplastic agent.

34. A pharmaceutical combination, comprising: a) an active **vitamin D** compound administered episodically; b) an antineoplastic agent co-administered with the **vitamin D** compound.

35. A kit comprising: a) an active **vitamin D** compound; b) an agent which an antineoplastic agent, a bone agent, and antihypercalcemic agent or combinations thereof; and c) instructions.

. .

37. The kit of claim 36 wherein the **vitamin D** compound and the antineoplastic agent are formulated for parenteral adminstration.

38. The kit of claim 36 wherein the **vitamin D** compound and the antineoplastic agent are manufactured physically separately and are intended for time-sequential co-administration.

39. The kit of claim 35 consisting essentially of a) an active **vitamin D** compound; b) an antineoplastic agent; and c) instructions effective to perform the method of claim 22.

40. The kit of claim 35 consisting essentially of a) an active **vitamin D** compound; b) an antineoplastic agent; c) an antihypercalcemic agent; and d) instructions effective to perform the method of claim 22.

. . .

41. The kit of claim 35, wherein the active **vitamin D** compound is present in dosage of between about 10 .mu.g and about 200 .mu.g.

L4 ANSWER 3 OF 12 USPATFULL
AN 2002:224270 USPATFULL
TI Methods of treating chronic inflammatory diseases using carbonyl trapping agents
IN Shapiro, Howard K., 214 Price Ave., Apt. F-32, Narberth, PA, United States 19072
PI US 6444221 B1 20020903
AI US 1999-416120 19991012 (9)
RLI Continuation-in-part of Ser. No. US 1995-473786, filed on 7 Jun 1995, now abandoned Continuation-in-part of Ser. No. US 1992-906909, filed on 30 Jun 1992, now abandoned
DT Utility
FS GRANTED
EXNAM Primary Examiner: Kulkosky, Peter F.; Assistant Examiner: Di Nola-Baron, Liliana
CLMN Number of Claims: 26
ECL Exemplary Claim: 1
DRWN 0 Drawing Figure(s); 0 Drawing Page(s)
LN.CNT 2400
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
SUMM **vitamin D.sub.2**, dosage range from 400 units daily to 40,000 units daily;
SUMM **vitamin D**.sub.3, dosage range from 400 units daily to 40,000 units daily;
SUMM **vitamin D.sub.4**, dosage range from 400 units daily to 40,000 units daily;
DETD Bernard, G R "N-Acetylcysteine in experimental and clinical acute **lung** injury" Am. J. Med. 91(Suppl 3C):54S-59S (1991)
DETD Passwater, R A The Antioxidants. The Nutrients that Guard the Body Against **Cancer**, Heart Disease, Arthritis and Allergies--and

Even Slow the Aging Process (New Canaan, Conn., Keats Publishing, 1985)

CLM What is claimed is:

. . . . ester monosodium salt; pyridoxine; pyridoxal; pyridoxal HCl; pyridoxal 5-phosphate; pyridoxal 5-phosphate calcium salt; pyridoxamine; pyridoxamine dihydrochloride; pyridoxamine phosphate; cyanocobalamin; co-methylcobalamin; **vitamin D.sub.2**; **vitamin D**.sub.3; **vitamin D.sub.4**; biotin; vitamin K.sub.1; vitamin K.sub.1 oxide; vitamins of the K.sub.2 series; vitamin K.sub.5; vitamin K.sub.5 hydrochloride; vitamin K.sub.6; vitamin K.sub.6. . .

. . . . ester monosodium salt; pyridoxine; pyridoxal; pyridoxal HCl; pyridoxal 5-phosphate; pyridoxal 5-phosphate calcium salt; pyridoxamine; pyridoxamine dihydrochloride; pyridoxamine phosphate; cyanocobalamin; co-methylcobalamin; **vitamin D.sub.2**; **vitamin D**.sub.3; **vitamin D.sub.4**; biotin; vitamin K.sub.1; vitamin K.sub.1 oxide; vitamins of the K.sub.2 series; vitamin K.sub.5; vitamin K.sub.5 hydrochloride; vitamin K.sub.6; vitamin K.sub.6. . .

. . . . ester monosodium salt; pyridoxine; pyridoxal; pyridoxal HCl; pyridoxal 5-phosphate; pyridoxal 5-phosphate calcium salt; pyridoxamine; pyridoxamine dihydrochloride; pyridoxamine phosphate; cyanocobalamin; co-methylcobalamin; **vitamin D.sub.2**; **vitamin D**.sub.3; **vitamin D.sub.4**; biotin; vitamin K.sub.1; vitamin K.sub.1 oxide; vitamins of the K.sub.2 series; vitamin K.sub.5; vitamin K.sub.5 hydrochloride; vitamin K.sub.6; vitamin K.sub.6. . .

. . . . ester monosodium salt; pyridoxine; pyridoxal; pyridoxal HCl; pyridoxal 5-phosphate; pyridoxal 5-phosphate calcium salt; pyridoxamine; pyridoxamine dihydrochloride; pyridoxamine phosphate; cyanocobalamin; co-methylcobalamin; **vitamin D.sub.2**; **vitamin D**.sub.3; **vitamin D.sub.4**; biotin; vitamin K.sub.1; vitamin K.sub.1 oxide; vitamins of the K.sub.2 series; vitamin K.sub.5; vitamin K.sub.5 hydrochloride; vitamin K.sub.6; vitamin K.sub.6. . .

. . . . ester monosodium salt; pyridoxine; pyridoxal; pyridoxal HCl; pyridoxal 5-phosphate; pyridoxal 5-phosphate calcium salt; pyridoxamine; pyridoxamine dihydrochloride; pyridoxamine phosphate; cyanocobalamin; co-methylcobalamin; **vitamin D.sub.2**; **vitamin D**.sub.3; **vitamin D.sub.4**; biotin; vitamin K.sub.1; vitamin K.sub.1 oxide; vitamins of the K.sub.2 series; vitamin K.sub.5; vitamin K.sub.5 hydrochloride; vitamin K.sub.6; vitamin K.sub.6. . .

L4 ANSWER 4 OF 12 USPATFULL

AN 2002:43583 USPATFULL

TI Method of treating hyperproliferative diseases using active **vitamin D** analogues

IN Bishop, Charles W., Madison, WI, UNITED STATES
Mazess, Richard B., Madison, WI, UNITED STATES

PA Bone Care International, Inc., Middleton, WI, UNITED STATES (U.S. corporation)

PI US 2002025950 A1 20020228
US 6503893 B2 20030107

AI US 2001-891814 A1 20010626 (9)

RLI Continuation-in-part of Ser. No. US 1998-596149, filed on 23 Feb 1998, PENDING Division of Ser. No. US 1996-781910, filed on 30 Dec 1996, GRANTED, Pat. No. US 5763429

DT Utility

FS APPLICATION

LREP MICHAEL BEST & FRIEDRICH, LLP, ONE SOUTH PINCKNEY STREET, P O BOX 1806, MADISON, WI, 53701

CLMN Number of Claims: 37

ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1129
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Method of treating hyperproliferative diseases using active **vitamin D** analogues
AB Methods for the utilization of hypocalcemic **vitamin D** analogs to inhibit the hyperproliferation of malignant or neoplastic cells without incidence of hypercalcemia.
SUMM . . . relates generally to a method of treating hyperproliferative diseases, and in particular, to the use of active forms of hypocalcemic **vitamin D** to inhibit the hyperproliferative cellular activity of these diseases and to promote differentiation of the cells.
SUMM [0004] Extensive research during the past two decades has established important biologic roles for **vitamin D** apart from its classic role in bone and mineral metabolism. Specific nuclear receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3, the hormonally active form of **vitamin D**, are present in cells from diverse organs not involved in calcium homeostasis. For example, specific, biologically active **vitamin D** receptors have been demonstrated in the human prostatic carcinoma cell line, LNCaP, (Miller et al., 52 **Cancer** Res. (1992) 515-520); **Vitamin D** receptors have also been described for many other neoplastic cells, e.g., carcinomas of the breast and the colon.
SUMM [0005] It has been reported that certain **vitamin D** compounds and analogues are potent inhibitors of malignant cell proliferation and are inducers/stimulators of cell differentiation. For example, U.S. Pat.. . . nonmalignant macrophages (monocytes), and are useful in the treatment of leukemia. Antiproliferative and differentiating actions of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues have been reported with respect to **cancer** cell lines. More recently, an association between **vitamin D** receptor gene polymorphism and **cancer** risk has been reported, suggesting that **vitamin D** receptors may have a role in the development, and possible treatment, of **cancer**.
SUMM [0006] These previous studies have focused exclusively on **vitamin D**.sub.3 compounds. Even though these compounds may indeed be highly effective in promoting differentiation in malignant cells in culture, their practical. . . blood calcium levels by virtue of their inherent calcemic activity. That is, the clinical use of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues as anticancer agents is precluded, or severely limited, by the risk of hypercalcemia. This indicates a need for compounds with greater specific activity and selectivity of action, i.e., **vitamin D** compounds with antiproliferative and differentiating effects but which have less calcemic activity.
SUMM . . . disease conditions such as those characterized by hyperproliferative cell growth and/or abnormal cell differentiation. The method includes use of active **vitamin D** compounds to inhibit abnormal cell growth and promote cell differentiation.
SUMM . . . inhibiting the hyperproliferative activity of neoplastic or hyperplastic cells, comprising treating the cells with an effective amount of a hypocalcemic **vitamin D** compound. The treating step includes inhibiting proliferation of, and inducing and enhancing differentiation in such cells.
SUMM [0009] The hypocalcemic **vitamin D** compounds of the present invention include **vitamin D** compounds having a hydrocarbon moiety substituted at the C-24 position on the sidechain of the molecule and a hydroxy group. . .
SUMM [0010] The **vitamin D** compound of the present invention is an active **vitamin D** and is suitably

represented by the formula (I) described hereafter. The compounds of formula (I) suitably include 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin. . .

SUMM [0011] Hypocalcemic **vitamin D** compounds are valuable for the treatment of breast and colon **cancer**, as well as other neoplasms such as pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell **cancer** of the head and neck, bladder, ovarian and cervical cancers, myeloid and lymphocyltic leukemia, lymphoma, hepatic tumors, medullary thyroid carcinoma,. . .

SUMM [0012] In accordance with the present invention, when effective amounts of hypocalcemic **vitamin D** compounds are administered to patients with **cancer** or neoplasms, the proliferative activity of the abnormal neoplastic cells is inhibited, reduced, or stabilized, and cell differentiation is induced, promoted or enhanced, with significantly less hypercalcemia and hypercalciuria than is observed after the same amount of an activated **vitamin D**.sub.3 (e.g., 1.alpha.-OH D.sub.3, 1.alpha.,25-(OH).sub.2 D.sub.3) is administered in previously known formulations. Thus, the compound in accordance with the present invention has an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues.

SUMM [0013] Accordingly, another aspect of the invention is a method of treating human **cancer** comprising administering to a subject who has **cancer** an effective amount of hypocalcemic **vitamin D** compound which has or attains through metabolism in vivo, a **vitamin D** receptor (VDR) binding affinity substantially equivalent to the binding affinity of 1.alpha.,25-dihydroxyvitamin D.sub.3 and a hypercalcemia risk substantially lower that that of 1.alpha.,25-dihydroxyvitamin D.sub.3, to inhibit, decrease or stabilize the cellular abnormal proliferative activity of the **cancer**.

SUMM [0014] For treatment for malignant conditions in accordance with the present invention, the hypocalcemic **vitamin D** compounds can be suitably administered alone as an active ingredient, as an antiproliferative agent in a pharmaceutical composition, or co-administered. . .

SUMM [0015] Further, included within the scope of the present invention is the co-administration of the **vitamin D** of formula (I) with a cytotoxic or anticancer agent. Such agents suitably include antimetabolites (e.g., 5-fluoro-uracil, methotrexate, fludarabine), antimicrotubule agents. . .

SUMM [0016] It is anticipated that the hypocalcemic **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

SUMM . . . administration of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. For example, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

SUMM [0018] In another aspect, the invention is a pharmaceutical composition which includes an anticancer agent which is an active **vitamin D** compound; an agent selected from the group consisting of (i) an anticancer agent, (ii) a bone agent, and combinations thereof;. . .

SUMM . . . diseased cells. The present invention provides a novel treatment of a patient suffering from a hyperproliferative disease such as prostatic **cancer** or prostatic hyperplasia with a hypocalcemic hydroxyvitamin D analogue. The **vitamin D** analogue is suitably a 1.alpha.-hydroxyvitamin D or a 24-hydroxyvitamin D compound. The hypocalcemic hydroxyvitamin D analogue represented by

formula (I). . . deleterious blood calcium levels and urine calcium levels, respectively. These attributes are achieved through specific chemical properties of the hypocalcemic **vitamin D** compounds as described.

SUMM [0022] In accordance with the present invention, when effective amounts of the hypocalcemic **vitamin D** compounds are administered to patients with **cancer** or hyperplasia, the proliferative activity of the abnormal cells is inhibited, maintained, or alleviated, and cell differentiation is induced, promoted or enhanced, with significantly less hypercalcemia and hypercalciuria than is observed after the same amount of activated **vitamin D**.sub.3 is administered in previously known formulations. Thus, the hypocalcemic **vitamin D** compounds of the present invention have an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues.

SUMM [0023] It is known that **vitamin D**.sub.3 must be hydroxylated in the C-1 and C-25 positions before it is activated, i.e., before it will produce a biological response. A similar metabolism appears to be required to activate other forms of **vitamin D**, e.g., **vitamin D.sub.2** and **vitamin D.sub.4**. Therefore, as used herein, the term "activated **vitamin D**" or "active **vitamin D**" is intended to refer to a **vitamin D** compound or analogue that has been hydroxylated in at least the C-1, C-24 or C-25 position of the molecule and either the compound itself or its metabolites in the case of a prodrug, such as 1.alpha.-hydroxyvitamin D.sub.2, binds the **vitamin D** receptor (VDR). For example, **vitamin D** "prodrugs" include compounds which are hydroxylated in the C-1 position. Such compounds undergo further hydroxylation in vivo and their metabolites. . .

SUMM [0024] The term "hypocalcemic **vitamin D** compound" is in reference to active **vitamin D** analogs which demonstrate reduced calcemic activity relative to the calcemic activity of 1.alpha.,25-dihydroxyvitamin D.sub.3. Such compounds include 24-hydroxyvitamin D compounds,. . .

SUMM [0027] The compound in accordance with the present invention is an active hypocalcemic **vitamin D** compound. Further, the active **vitamin D** in accordance with the present invention may have an unsaturated sidechain, e.g., there is suitably a double bond between C-22. . .

SUMM [0034] The hypocalcemic **vitamin D** compounds of the present invention are those that have effective antiproliferative and cell differentiation activity (i.e., reversal of malignant transformation),. . . to malignant or other hyperproliferative cells without significantly altering calcium metabolism. This selectivity and specificity of action makes the hypocalcemic **vitamin D** compounds useful and preferred agents for safely inhibiting hyperproliferation and promoting malignant or hyperplastic cell differentiation. The compounds of the present invention, thus, overcome the shortcomings of the known active **vitamin D**.sub.3 compounds described above, and can be considered preferred agents for the control and treatment of malignant diseases such breast, prostate, testicular and colon **cancer**, as well as other neoplasms such as pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . medullary thyroid carcinoma, multiple myeloma, melanoma, retinoblastoma, and sarcomas of the soft tissue and bone, i.e. neoplasms that express a **vitamin D** receptor.

SUMM . . . hyperproliferative cells, (i.e., inhibiting their

hyperproliferative activity and/or inducing and enhancing their differentiation) with an effective amount of a hypocalcemic **vitamin D** compound. The effective dosage amount on a daily basis per kilogram of body weight of the patient ranges from about. . . dose is given. The compounds in accordance with the present invention are administered in an amount that raises a serum **vitamin D** level to a supraphysiological level for a sufficient period of time to induce differentiation or regression of a tumor or. . .

SUMM [0036] The compounds of formula (I) are valuable for the treatment of **cancer** and neoplasms in a patient suffering therefrom. In particular, the invention is a method for treating a patient suffering from the hyperproliferative cellular effects of **cancer** and other neoplasms by administering to the patient a therapeutically effective amount of a compound of formula (I), which is. . .

SUMM . . . the compounds of formula (I) have been studied and compared to that of 1.alpha.,25-dihydroxyvitamin D.sub.3, the active hormonal form of **vitamin D** and the standard against which all **vitamin D** compounds and analogues are measured. For example, it has been found that the **vitamin D** receptor (VDR) binding affinities of the compounds of formula (I), or their active metabolites, are substantially equivalent to (i.e., equal. . .

SUMM . . . At the same time, it has been found that compounds of formula (I) are significantly less toxic than their corresponding **vitamin D**.sub.3 analogues. For example, in parent co-pending application, Ser. No. 08/265,438, the disclosure of which is incorporated herein by reference, the. . .

SUMM . . . in pharmaceutical compositions having reduced side effects and low toxicity as compared with the known analogues of active forms of **vitamin D**.sub.3.

SUMM . . . conventional methods of pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans. For example, the hypercalcemic **vitamin D** compounds of the present invention can be employed in admixtures with conventional excipients, e.g., pharmaceutically acceptable carrier substances suitable for. . .

SUMM . . . wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or one or more other active compounds, for example, **vitamin D**.sub.3 and its 1.alpha.-hydroxylated metabolites, conjugated estrogens or their equivalents, anti-estrogens, calcitonin, biphosphonates, calcium supplements, cobalamin, pertussis toxin and boron.

SUMM . . . about 0.01 .mu.g to about 50 .mu.g per gram of composition. For treatment of cancers, the dosage of the hypocalcemic **vitamin D** compound in a locally applied composition generally is about 0.01 .mu.g to 100 .mu.g per gram composition.

SUMM . . . administration of the pharmaceutical compositions of the present invention is preferred. The dosage of the compounds for the treatment of **cancer** or neoplasms according to this invention generally is about 0.01 to about 2.0 .mu.g/kg/day, preferably about 0.01 to about 1.0 .mu.g/kg/day. As noted above, dosing of the hypocalcemic **vitamin D** compounds in accordance with the present invention can be done on an episodic basis, in which higher does can be. . .

SUMM [0053] Further, included within the scope of the present invention is a method of co-administration of hypercalemic **vitamin D** compounds with an anticancer or antineoplastic agent. Such agents may suitably include antimetabolites (e.g., 5-fluoro-uracil, methotrexate, fludarabine), antimicrotubule agents (e.g.,. . . adriamycin, daunomycin), topoisomerase inhibitiors (e.g., etoposide, camptothecins) or any other antineoplastic agents. (estramustine phosphate,

prednimustine). It is anticipated that hypercalcemic **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

SUMM . . . other at a later time, typically within a week. An example of a suitable co-administration regimen is where a hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of a cytotoxic agent.

SUMM [0055] Also included within the scope of the present invention is the co-administration of effective dosages of hypercalcemic **vitamin D** compounds in conjunction with administration of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. For example, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

DETD [0058] The affinity of 1.alpha.,24-(OH).sub.2D.sub.2 for the mammalian **vitamin D** receptor (VDR) was assessed using a commercially available kit of bovine thymus VDR and standard 1,25-(OH).sub.2D.sub.3 solutions from Incstar (Stillwater,. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0059] The VDR affinity binding of 1.alpha.,24-(OH).sub.2D.sub.4 was investigated. The 1.alpha.,24-(OH).sub.2D.sub.4 was incubated with **vitamin D** receptor and radiolabeled tracer 1.alpha.,25-(OH).sub.2D.sub.3. After incubation, the amount of radioactivity bound to the receptor was determined and compared with. .

DETD [0060] These results show that 1.alpha.,24-(OH).sub.2D.sub.4 binds slightly less tightly to the **vitamin D** receptor than does 1.alpha.,25-(OH).sub.2D.sub.3. Such data mean that 1.alpha.,24-(OH).sub.2D.sub.4 has high affinity for the VDR and significant biological activity, similar. . .

DETD . . . results are surprising and unexpected in view of the prior art. They are contrary to the normative wisdom in the **vitamin D** art regarding the very low degree of biological activity of **vitamin D.sub.4** compounds.

DETD [0062] VDR binding of **vitamin D** compounds by prostate cells is demonstrated using the techniques of Skowronski et al., 136 Endocrinology (1995) 20-26, which is incorporated. . .

DETD 1.alpha.,24-dihydroxy **Vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0063] The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,24-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. . .

DETD [0064] The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,25-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. . .

DETD 1.alpha.,24-dihydroxy **Vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0065] Using the plasmids p(CT4).sup.4TKGH, a **vitamin D** receptor (VDR)-expressing plasmid, and pSG5-hVDR1/3, a plasmid containing a Growth Hormone (GH) gene, under the control of a **vitamin D**-responsive element (VDRE), experiments were conducted to explore the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to induce **vitamin D**-dependent growth hormone acting as a reporter gene compared to that of 1.alpha.,25-(OH).sub.2D.sub.3. Cells in culture were transfected with these two plasmids. One plasmid contained the gene for Growth Hormone (GH) under the control of the

vitamin D responsive element (VDRE) and the other plasmid contained the structural gene for the **vitamin D** receptor (VDR). These transfected cultures were incubated with 1.alpha.,24-(OH).sub.2D.sub.4 or 1.alpha.,25-(OH).sub.2D.sub.3, and the production of growth hormone was measured. Table 2 below shows the results of this assay:

TABLE 2

Induction of Growth Hormone by **Vitamin D** Compounds

Compound	Concentration Used (M)	Growth Hormone Induction (ng/ml)
1,25-(OH).sub.2D.sub.3	.sup. 1 .times. 10.sup.-10	39
1,25-(OH).sub.2D.sub.3	.sup. 5 .times. 10.sup.-10	248
1,24-(OH).sub.2D.sub.4	.sup. 5. . .	

DETD [0066] These data show that the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to stimulate **vitamin D**-dependent growth hormone is nearly equivalent to that of 1.alpha.,25-(OH).sub.2D.sub.3. Such results are truly surprising and would not have been expected. . .

DETD 1.alpha.,24(S)-dihydroxyvitamin D.sub.2 and 1.alpha.,24(R)-dihydroxy-**vitamin D.sub.2** [1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2]

DETD . . . was conducted to compare the biological activity in vitro of chemically synthesized 1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2, with 1.alpha.,25-(OH).sub.2D.sub.3 and 25-OH-D.sub.3. The **vitamin D**-dependent transcriptional activation model system was used in which plasmids pSG5-hVDR1/3 and p(CT4).sup.4TKGH were co-transfected into Green monkey kidney, COS-1 cells.

DETD [0068] Transfected cells were incubated with **vitamin D** metabolites and growth hormone production was measured. As shown in Table 3, both 1.alpha.,24(S)-(OH).sub.2D.sub.2 and its epimer, 1.alpha.,24(R)-(OH).sub.2D.sub.2, had significantly more activity in this system than 25-OH-D.sub.3, with 1.alpha.,24(S)-(OH).sub.2D.sub.2 having nearly the same activity as 1.alpha.,25-(OH).sub.2D.sub.3.

TABLE 3

Vitamin D-Inducible Growth Hormone Production In Transfected COS-1 Cells

Inducer	Molar Concentration. . .	Total GH Production*	Vitamin DCInducible Growth Hormone Production Net vitamin DCinducible GH-production

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 to 10.sup.-7 M. Medium containing test analogue or vehicle is replaced every three days.. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0070] The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly fewer cells than the

control cultures.

DETD [0071] The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue, 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. After 6-7 days, the medium is removed and stored at. . .

DETD [0074] The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,24-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD [0075] The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,25-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD [0076] Patients with a known **vitamin D** receptor positive tumor (e.g., adenocarcinoma of the prostate, breast, **lung**, colon or pancreas, or transitional cell carcinoma of the bladder, or melanoma) participate in an open-label study of a hypocalcemic **vitamin D** compound in accordance with the present invention. Patients are placed on a reduced calcium diet prior to treatment, to help minimize intestinal absorption and allow ever higher doses of the hypocalcemic **vitamin D**. This reduced calcium diet may be continued for the duration of treatment, and for one week after the last dose. . . of the 1.alpha.,24(S)-dihydroxyvitamin D.sub.2. The diet ideally restricts daily calcium intake to 400-500 mg. Patients also discontinue use of any **vitamin D** supplements or **vitamin D** replacement therapies. Each patient is also asked to drink 4-6 cups of fluid more than usual intake to assure adequate. . .

DETD Treatment of Prostate **Cancer** with 1.alpha.,24-dihydroxyvitamin D.sub.2 [(1.alpha.,24-(OH).sub.2D.sub.2]

DETD [0080] Patients with advanced androgen-independent prostate **cancer** participate in an open-labeled study of 1.alpha.,24-(OH).sub.2D.sub.2. Qualified patients are at least 40 years old, exhibit histologic evidence of adenocarcinoma. . . patients begin a course of therapy with oral 1.alpha.,24-(OH).sub.2D.sub.2 lasting 26 weeks, while discontinuing any previous use of calcium supplements, **vitamin D** supplements, and **vitamin D** hormone replacement therapies. During treatment, the patients are monitored at regular intervals for: (1) hypercalcemia, hyperphosphatemia, hypercalciuria, hyperphosphaturia and other. . .

DETD Treatment of Prostate **Cancer** with 1.alpha.-hydroxyvitamin D.sub.2 [1.alpha.-OH-D.sub.2]

DETD [0084] The study of Example 14 is repeated for the active **vitamin D** compound, 1.alpha.-OH-D.sub.2. The results of the phase one study indicate that patients treated with the MTD of 1.alpha.-OH-D.sub.2 for at. . .

DETD Treatment of Liver **Cancer**

CLM What is claimed is:

. . . antiproliferative amount of a hypocalcemic hydroxyvitamin D compound having a hydrocarbon moiety at the C.sub.24 position, the cells expressing a **vitamin D** receptor.

2. The method of claim 1, wherein the cells are cancers of the breast,

colon, **lung**, neck and head, pancreas, endometrium, bladder, cervix, testes, ovaries, squamous cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma,. . .

3. The method of claim 1, wherein the hypocalcemic **vitamin D** is a compound represented by formula (I) ##STR4## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon bond, thus. . .

4. A method in accordance with claim 1 wherein the hypocalcemic **vitamin D** compound is a compound of formula II ##STR5## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon bond, thus. . .

5. A method in accordance with claim 1, wherein the hypocalcemic **vitamin D** compound is a compound of formula III: ##STR6## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon bond, thus. . .

7. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is administered in a daily regimen or an episodic regimen.

9. A method in accordance with claim 7, wherein the hypocalcemic **vitamin D** compound is administered daily at a dose of about 10 to 100 .mu.g/day.

10. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is administered orally, is administered intravenously, is directly injected to a **cancer** site or is regionally delivered to a **cancer** site.

11. A method in accordance with claim 10, wherein the hypocalcemic **vitamin D** compound is administered orally.

12. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is co-administered with a cytotoxic agent.

25. A method of treating a human to alleviate the pathological effects of breast **cancer**, colon **cancer**, testicular **cancer**, pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . .

26. A method of claim 25, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula (III) ##STR7## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon. . .

. . . a disease in need of treatment by a cytotoxic agent, comprising administering to the patient a therapeutic amount of hypocalcemic **vitamin D** compound and the cytotoxic agent.

30. A method in accordance with claim 29, wherein the hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of the cytotoxic agent.

31. A method in accordance with claim 29, wherein the hypocalcemic **vitamin D** compound is administered 2 to 4 days prior to administration of the cytotoxic agent.

32. A method of claim 29, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula (III) ##STR8## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon. . .

33. The method of claim 32, wherein said therapeutic amount of the **vitamin D** compound is 0.01 .mu.g/kg/day to 2.0 .mu.g/kg/day.

. . . of inducing differentiation in malignant or neoplastic cells, comprising treating to the cells with a prodifferentiative amount of a hypocalcemic **vitamin D** compound.

37. A method of treating in a subject tumor or neoplasm that expresses a **vitamin D** receptor, comprising administering to the subject an effective amount of hypocalcemic **vitamin D** compound sufficient to raise a blood level of **vitamin D** to a sufficiently supraphysiological level for a sufficient period of time to inhibit growth of the tumor or neoplasm without. . .

L4 ANSWER 5 OF 12 USPATFULL
AN 2002:32553 USPATFULL
TI Method of inhibiting angiogenesis using active **vitamin D** analogues
IN Bishop, Charles W., Madison, WI, UNITED STATES
Mazess, Richard B., Madison, WI, UNITED STATES
PA Bone Care International, Inc., Middleton, WI, UNITED STATES (U.S. corporation)
PI US 2002019375 A1 20020214
US 6573256 B2 20030603
AI US 2001-891805 A1 20010626 (9)
RLI Continuation-in-part of Ser. No. US 1998-596149, filed on 23 Feb 1998, PENDING Division of Ser. No. US 1996-781910, filed on 30 Dec 1996, GRANTED, Pat. No. US 5763429
DT Utility
FS APPLICATION
LREP MICHAEL BEST & FRIEDRICH, LLP, ONE SOUTH PINCKNEY STREET, P O BOX 1806, MADISON, WI, 53701
CLMN Number of Claims: 34
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1134
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Method of inhibiting angiogenesis using active **vitamin D** analogues
AB Methods utilizing active **vitamin D** analogs for the inhibition of angiogenesis associated with malignant and neoplasmic cells. Methods comprise the application of an effective amount of a hypocalcemic **vitamin D** compound to inhibit the angiogenesis of malignant cells, inducing the apoptosis of malignant cells, and regressing the growth of tumorous. . .
SUMM . . . of inhibiting angiogenesis associated with the hyperproliferation of malignant cells, and in particular, to the use of active forms of **vitamin D** to inhibit angiogenesis of malignant cells.
SUMM [0004] Extensive research during the past two decades has established important biologic roles for **vitamin D** apart from its classic role in bone and mineral metabolism. Specific nuclear receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3, the hormonally active form of **vitamin D**, are present in cells from diverse organs not involved in calcium homeostasis. For example, specific, biologically active **vitamin D** receptors have been demonstrated in the human prostatic carcinoma cell line, LNCaP, (Miller et al., 52 **Cancer** Res. (1992) 515-520); **Vitamin D** receptors have also been described for many other neoplastic cells, e.g., carcinomas of the breast and the colon.
SUMM [0005] It has been reported that certain **vitamin D**

compounds and analogues are potent inhibitors of malignant cell proliferation and are inducers/stimulators of cell differentiation. For example, U.S. Pat.. . . nonmalignant macrophages (monocytes), and are useful in the treatment of leukemia. Antiproliferative and differentiating actions of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues have been reported with respect to **cancer** cell lines. More recently, an association between **vitamin D** receptor gene polymorphism and **cancer** risk has been reported, suggesting that **vitamin D** receptors may have a role in the development, and possible treatment, of **cancer**.

SUMM [0006] These previous studies have focused exclusively on **vitamin D**.sub.3 compounds. Even though these compounds may indeed be highly effective in promoting differentiation in malignant cells in culture, their practical. . . blood calcium levels by virtue of their inherent calcemic activity. That is, the clinical use of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues as anticancer agents is precluded, or severely limited, by the risk of hypercalcemia.

SUMM . . . the regression was due to the induction of apoptosis within the cell population. As mentioned earlier however, use of such **vitamin D**.sub.3 analogs as anticancer agents is limited due to the inherent calcemic activity of the compounds. Therefore a need exists for. . .

SUMM . . . The present invention provides a method of inhibiting angiogenesis associated with malignant cells. The method includes use of hypocalcemic active **vitamin D** compounds to inhibit angiogenesis. The present invention also provides a method of inducing the apoptosis of **cancer** cells by the use of active **vitamin D** compounds, and includes a method for treating **cancer** by regressing tumor cells by the use of active **vitamin D** compounds.

SUMM . . . a method of inhibiting angiogenesis associated with malignant cells, comprising treating the cells with an effective amount of a hypocalcemic **vitamin D** compound. The hypocalcemic **vitamin D** compound of the present invention include hypocalcemic **vitamin D** compounds having a hydrocarbon moiety substituted at the C-24 position on the sidechain of the molecule and having a hydroxyl. . .

SUMM [0011] The hypocalcemic **vitamin D** compound is an active **vitamin D** and is suitably represented by the formula (I) described hereafter. Suitable compounds of formula (I), are 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4,. . .

SUMM [0013] In another aspect of the invention, the apoptosis of **cancer** cells is accomplished by a method comprising, administering to patients an effective amount of a hypocalcemic **vitamin D** compound to induce the apoptosis of **cancer** cells.

SUMM [0014] In yet another aspect of the invention, a method for treating **cancer** by regressing tumor cells is disclosed, comprising administering to patients an effective amount of a hypocalcemic **vitamin D** compound to induce the regression of **cancer** cells.

SUMM [0015] In accordance with the present invention, when effective amounts of the hypocalcemic **vitamin D** compounds are administered to patients with **cancer** or neoplasms, the proliferative activity of the abnormal neoplastic cells is inhibited or maintained, and cell differentiation is induced, promoted or enhanced, with significantly less hypercalcemia and hypercalciuria than is observed after the same amount of an activated **vitamin D**.sub.3 (e.g., 1.alpha.-OH D.sub.3, 1.alpha.,25-(OH).sub.2

D.sub.3) is administered in previously known formulations. Thus, the compound in accordance with the present invention has an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues. Furthermore, the compounds of the present invention can be administered in doses significantly higher than that of active **vitamin D**.sub.3 analogs due to their lower calcemic effect.

SUMM [0016] Accordingly, another aspect of the invention is a method of treating human **cancer** comprising administering to a subject who has **cancer** an effective amount of active **vitamin D** compound which has, attained through metabolism in vivo, a **vitamin D** receptor (VDR) binding affinity substantially equivalent to the binding affinity of 1.alpha.,25-dihydroxyvitamin D.sub.3 and a hypercalcemia risk substantially lower that that of 1.alpha.,25-dihydroxyvitamin D.sub.3, to decrease or stabilize the cellular abnormal proliferative activity of the **cancer**.

SUMM [0017] For treatment for malignant conditions in accordance with the present invention, the active **vitamin D** is suitably administered alone as an active ingredient in a pharmaceutical composition, or is co-administered with an anticancer agent.

SUMM [0018] Further, included within the scope of the present invention is the co-administration of a hypocalcemic **vitamin D** compound with a cytotoxic or anticancer agent. Such agents suitably include antimetabolites (e.g., 5-fluoro-uracil, methotrexate, fludarabine), antimicrotubule agents (e.g., vincristine,. . .

SUMM [0019] It is anticipated that the active **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

SUMM [0020] Also included within the scope of the present invention is the co-administration of effective dosages of a hypocalcemic **vitamin D** compound in conjunction with administration of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. For example, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

SUMM [0021] In another aspect, the invention is a pharmaceutical composition which includes an anticancer agent which is an active **vitamin D** compound; an agent selected from the group consisting of (i) an anticancer agent, (ii) a bone agent, and combinations thereof;. . .

DETD . . . present invention provides a novel inhibition of angiogenesis of a patient suffering from a hyperproliferative disease with an active hypocalcemic **vitamin D** compound. The active **vitamin D** analogue is suitably a hydroxyvitamin D compound e.g. a 1.alpha.-hydroxy **vitamin D** or a 24-hydroxy **vitamin D**, and is suitably represented by formula (I) as described hereinbelow. The active **vitamin D** analogue is provided to the patient without causing dose-limiting hypercalcemia and hypercalciuria, i.e., unphysiologically high and deleterious blood calcium levels. . . in fact reduces the hypercalcemia caused by the malignancy. These attributes are achieved through specific chemical properties of the hypocalcemic **vitamin D** compounds as described.

DETD [0025] In accordance with the present invention, when effective amounts of the analogues of the hypocalcemic **vitamin D** compound are administered to patients with malignant diseases, the angiogenesis of cancerous cells is inhibited, tumorous cells are regressed, cancerous. . . is induced, promoted or enhanced, with significantly less hypercalcemia and hypercalciuria than is observed after the same amount of activated **vitamin D**.sub.3 is administered in previously known formulations. Thus, the hypocalcemic

vitamin D compounds of the present invention have an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues.

DETD [0026] It is known that **vitamin D**.sub.3 must be hydroxylated in the C-1 and C-25 positions before it is activated, i.e., before it will produce a biological response. A similar metabolism appears to be required to activate other forms of **vitamin D**, e.g., **vitamin D.sub.2** and **vitamin D.sub.4**. Therefore, as used herein, the term "activated **vitamin D**" or "active **vitamin D**" is intended to refer to a **vitamin D** compound or analogue that has been hydroxylated in at least the C-1, C-24 or C-25 position of the molecule and either the compound itself or its metabolites in the case of a prodrug, such as 1.alpha.-hydroxyvitamin D.sub.2, binds the **vitamin D** receptor (VDR). For example, "prodrugs" include **vitamin D** compounds which are hydroxylated in the C-1 position. Such compounds undergo further hydroxylation in vivo and their metabolites bind the. . .

DETD [0027] The term "hypocalcemic **vitamin D** compound" is in reference to active **vitamin D** analogs which demonstrate hypocalcemic activity, i.e., substantially less calcemic activity relative to the calcemic activity of 1.alpha.,25-dihydroxy **vitamin D**.sub.3. Such compounds include 24-hydroxyvitamin D compounds, 25-hydroxyvitamin D compounds and 1.alpha.-hydroxyvitamin D compounds.

DETD [0030] The compound in accordance with the present invention is an active hypocalcemic **vitamin D** compound. The active **vitamin D** provided is such that the compound has a hydrocarbon moiety at the C-24 position, e.g. a lower alkyl, alkenyl or acyl group as the C-24 position. Further, the active **vitamin D** in accordance with the present invention may have an unsaturated sidechain, e.g., there is suitably a double bond between C-22. . .

DETD [0037] The hypocalcemic **vitamin D** compounds of formula (I) of the present invention are those that have an effective inhibition effect on the angiogenesis of. . . to malignant or other hyperproliferative cells without significantly altering calcium metabolism. This selectivity and specificity of action makes the hypocalcemic **vitamin D** compounds useful and preferred agents for safely inhibiting angiogenesis of hyperproliferative cells. The compounds of the present invention, thus, overcome the shortcomings of the known active **vitamin D**.sub.3 compounds described above, and can be considered preferred agents for the control angiogenesis of malignant diseases such breast, colon, testicular and prostate **cancer**, as well as other neoplasms such as pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . medullary thyroid carcinoma, multiple myeloma, melanoma, retinoblastoma, and sarcomas of the soft tissue and bone, i.e., neoplasms that express a **vitamin D** receptor.

DETD [0038] Suitable active **vitamin D** compounds of formula (I) include: 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin D.sub.2, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.-hydroxyvitamin D.sub.2, and 1.alpha.-hydroxyvitamin D.sub.4. Among those. . .

DETD . . . of malignant cells as well as other hyperproliferative cells such as psoriatic cells with an effective amount of a hypocalcemic **vitamin D** compound. The effective dosage amount on a daily basis per kilogram of body weight of the patient ranges from

about. . . dose is given. The compounds in accordance with the present invention are administered in an amount that raises a serum **vitamin D** level to a supraphysiological level for a sufficient time to inhibit angiogenesis or induce the hypercalcemic properties of the compounds. . .

DETD [0040] The compounds of formula (I) are valuable for the inhibition of angiogenesis of **cancer** and neoplasms in a patient suffering therefrom. In particular, the invention is a method for treating a patient suffering from the hyperproliferative cellular effects of **cancer** and othe neoplasms by administering to the patient a therapeutically effective amount of a compound of formula (I), which is. . . suitably 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin D.sub.2, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.-hydroxyvitamin D.sub.2, and 1.alpha.-hydroxyvitamin D.sub.4, sufficient to inhibit antiogenesis of the **cancer** neoplasms. Among those compounds of formula (I) that have a chiral center in the sidechain, such as at C-24, it. . .

DETD . . . the compounds of formula (I) have been studied and compared to that of 1.alpha.,25-dihydroxyvitamin D.sub.3, the active hormonal form of **vitamin D** and the standard against which all **vitamin D** compounds and analogues are measured. For example, it has been found that the **vitamin D** receptor (VDR) binding affinities of the compounds of formula (I), or their active metabolites, are substantially equivalent to (i.e., equal. . .

DETD . . . At the same time, it has been found that compounds of formula (I) are significantly less toxic than their corresponding **vitamin D**.sub.3 analogues. For example, in parent co-pending application, Ser. No. 08/265,438, the disclosure of which is incorporated herein by reference, the. . .

DETD [0044] The hypocalcemic **vitamin D** compounds are useful as active compounds or ingredients in pharmaceutical compositions having reduced side effects and low toxicity as compared with the known analogues of active forms of **vitamin D**.sub.3.

DETD . . . conventional methods of pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans. For example, the hypocalcemic **vitamin D** compounds of the present invention can be employed in admixtures with conventional excipients, e.g., pharmaceutically acceptable carrier substances suitable for. . .

DETD . . . wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or one or more other active compounds, for example, **vitamin D**.sub.3 and its 1.alpha.-hydroxylated metabolites, conjugated estrogens or their equivalents, anti-estrogens, calcitonin, biphosphonates, calcium supplements, cobalamin, pertussis toxin and boron.

DETD . . . is about 0.01 .mu.g to about 50 .mu.g per gram of composition. For treatment of cancers, the dosage of hypocalcemic **vitamin D** compound in a locally applied composition generally is about 0.01 .mu.g to 100 .mu.g per gram composition.

DETD . . . administration of the pharmaceutical compositions of the present invention is preferred. The dosage of the compounds for the treatment of **cancer** or neoplasms according to this invention generally is about 0.01 to about 2.0 .mu.g/kg/day, preferably about 0.01 to about 1.0 .mu.g/kg/day. As noted above, dosing of the hypercalcemia **vitamin D** compounds in accordance with the present invention can be done on an episodic basis in which higher doses can be. . .

DETD [0057] Further, included within the scope of the present invention is the co-administration of hypocalcemic **vitamin D** compound with an anticancer agent, e.g., a cytotoxic agent, Such agents

suitably include antimetabolites (e.g., 5-fluoro-uracil, methotrexate, fludarabine), antimicrotubule agents. . . daunomycin), topoisomerase inhibitors (e.g., etoposide, camptothecins) or any other cytotoxic agents. (estramustine phosphate, prednimustine). It is anticipated that the hypocalcemic **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

DETD . . . other at a later time, typically within a week. An example of a suitable co-administration regimen is where a hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of a cytotoxic agent.

DETD [0059] Also included within the scope of the present invention is the co-administration of effective dosages of the hypocalcemic **vitamin D** compounds in conjunction with administration of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. For example, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

DETD [0062] The affinity of 1.alpha.,24-(OH).sub.2D.sub.2 for the mammalian **vitamin D** receptor (VDR) was assessed using a commercially available kit of bovine thymus VDR and standard 1 ,25-(OH).sub.2D.sub.3 solutions from Incstar. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub. 4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0063] The VDR affinity binding of 1.alpha.,24-(OH).sub.2D.sub.4 was investigated. The 1.alpha.,24-(OH).sub.2D.sub.4 was incubated with **vitamin D** receptor and radiolabeled tracer 1.alpha.,25-(OH).sub.2D.sub.3. After incubation, the amount of radioactivity bound to the receptor was determined and compared with. .

DETD [0064] These results show that 1.alpha.,24-(OH).sub.2D.sub.4 binds slightly less tightly to the **vitamin D** receptor than does 1.alpha.,25-(OH).sub.2D.sub.3. Such data mean that 1.alpha.,24-(OH).sub.2D.sub.4 has high affinity for the VDR and significant biological activity, similar. . .

DETD . . . results are surprising and unexpected in view of the prior art. They are contrary to the normative wisdom in the **vitamin D** art regarding the very low degree of biological activity of **vitamin D.sub.4** compounds.

DETD [0066] VDR binding of **vitamin D** compounds by prostate cells is demonstrated using the techniques of Skowronski et al., 136 Endocrinology (1995) 20-26, which is incorporated. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub. 4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0067] The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,24-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. . .

DETD [0068] The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,25-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub. 4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0069] Using the plasmids p(CT4).sup.4TKGH, a **vitamin D** receptor (VDR)-expressing plasmid, and pSG5-hVDR1/3, a plasmid containing a Growth Hormone (GH) gene, under the control of a **vitamin D**-responsive element (VDRE), experiments were conducted to explore the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to

induce **vitamin D**-dependent growth hormone acting as a reporter gene compared to that of 1.alpha.,25-(OH).sub.2D.sub.3. Cells in culture were transfected with these two plasmids. One plasmid contained the gene for Growth Hormone (GH) under the control of the **vitamin D** responsive element (VDRE) and the other plasmid contained the structural gene for the **vitamin D** receptor (VDR). These transfected cultures were incubated with 1.alpha.,24-(OH).sub.2D.sub.4 or 1.alpha.,25-(OH).sub.2D.sub.3, and the production of growth hormone was measured. Table 2 below shows the results of this assay:

TABLE 2

Induction of Growth Hormone by **Vitamin D** Compounds

Compound	Concentration Used (M)	Growth Hormone Induction (ng/ml)
1,25-(OH).sub.2D.sub.3	1 .times. 10.sup.-10	39
1,25-(OH).sub.2D.sub.3	5 .times. 10.sup.-10	248
1,24-(OH).sub.2D.sub.4	5 .times. 10.sup.-10	165
1,24-(OH).sub.2D.sub.4.	. .	

DETD [0070] These data show that the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to stimulate **vitamin D**-dependent growth hormone is nearly equivalent to that of 1.alpha.,25-(OH).sub.2D.sub.3. Such results are truly surprising and would not have been expected. . .

DETD 1.alpha.,24(S)-dihydroxyvitamin D.sub.2 and 1.alpha.,24(R)-dihydroxy-**vitamin D.sub.2** [1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2]

DETD . . . was conducted to compare the biological activity in vitro of chemically synthesized 1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2, with 1.alpha.,25-(OH).sub.2D.sub.3 and 25-OH-D.sub.3. The **vitamin D**-dependent transcriptional activation model system was used in which plasmids pSG5-hVDR1/3 and p(CT4).sup.4TKGH were co-transfected into Green monkey kidney, COS-1 cells.

DETD [0072] Transfected cells were incubated with **vitamin D** metabolites and growth hormone production was measured. As shown in Table 3, both 1.alpha.,24(S)-(OH).sub.2D.sub.2 and its epimer, 1.alpha.,24(R)-(OH).sub.2D.sub.2, had significantly more activity in this system than 25-OH-D.sub.3, with 1.alpha.,24(S)-(OH).sub.2D.sub.2 having nearly the same activity as 1.alpha.,25-(OH).sub.2D.sub.3.

TABLE 3

Vitamin D-Inducible Growth Hormone Production In Transfected COS-1 Cells

Inducer	Molar Concentration. . .	Vitamin DCInducible Growth Hormone Production: Total GH Production*	Net vitamin DCinducible GH-production

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. Medium containing test analogue or vehicle is replaced every three. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0074] The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with

1.alpha.,24-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD [0075] The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue, 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.31 7 M. After 6-7 days, the medium is removed and stored. . .

DETD [0078] The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,24-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD [0079] The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,25-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD [0080] A **vitamin D** compound of formula (I) inhibition of VEGF-induced endothelial cell proliferation is demonstrated using the techniques of Mantell et al., Circulation. . .

CLM What is claimed is:

. . . inhibiting angiogenesis associated with malignant or neoplastic cells, comprising treating the cells with an effective amount of a hypocalcemic hydroxy **vitamin D** compound having a hydrocarbon moiety at the C.sub.24 position, the cells being cancers of the breast, colon, prostate, testes, **lung**, neck and head, pancreas, endometrium, bladder, cervix, ovaries, squamous cell carcinomas, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma, melanoma,. . .

3. A method in accordance with claim 2, wherein the hypocalcemic **vitamin D** compound is represented by formula II: ##STR5## wherein A.sup.1 and A.sup.2each are hydrogen or a carbon-carbon bond, thus forming a. . .

4. The method of claim 2, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound is represented by formula III: ##STR6## wherein A.sup.1 and A.sup.2 each are hydrogen or a. . .

6. A method in accordance with claim 2, wherein a dosing regimen for the hypocalcemic **vitamin D** compound is a daily regimen or an episodic regimen.

8. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is administered daily at a dose of about 10 to 100 .mu.g/day.

9. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is administered orally, is administered intravenously, is injected directly into a **cancer** site, or is regionally delivered to a **cancer** site.

10. A method in accordance with claim 9, wherein the hypocalcemic **vitamin D** compound is administered orally.

11. A method in accordance with claim 2, wherein the hypocalcemic **vitamin D** compound is co-administered with a cytotoxic agent.

22. A method of treating a human to inhibit angiogenesis associated with breast **cancer**, colon **cancer**, prostate **cancer** , testicular **cancer**, pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . retinoblastoma or sarcomas of the soft tissue and bone, comprising administering to the human an effective amount of a hypocalcemic **vitamin D** compound.

23. A method of claim 22, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula III: ##STR7## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon. . .

. . . A method of treating a human to inhibit angiogenesis associated with malignant cells, comprising administering to the patient a hypocalcemic **vitamin D** compound and a cytotoxic agent.

27. A method in accordance with claim 26, wherein the hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of the cytotoxic agent.

28. A method in accordance with claim 26, wherein the hypocalcemic **vitamin D** compound is administered 2 to 4 days prior to administration of the cytotoxic agent.

29. A method of claim 26, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula III: ##STR8## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon. . .

. . . method of inducing apoptosis of malignant or neoplastic cells, comprising treating the cells with an effective amount of a hypocalcemic **vitamin D** compound, the cells being cancers of the breast, colon, prostate, testes, **lung**, neck and head, pancreas, endometrium, bladder, cervix, ovaries, squamous cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma, melanoma,. . .

. . . A method of inducing the regression of tumor cells comprising treating the cells with an effective amount of a hypocalcemic **vitamin D** compound, the cells being cancers of the breast, colon, prostate, testes, **lung**, neck and head, pancreas, endometrium, bladder, cervix, ovaries, squamous cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma, melanoma,. . .

L4 ANSWER 6 OF 12 USPATFULL
AN 2002:17285 USPATFULL
TI Method of treating malignancy associated hypercalcemia using active **vitamin D** analogues
IN Bishop, Charles W., Madison, WI, UNITED STATES
Mazess, Richard B., Madison, WI, UNITED STATES
PA Bone Care International, Inc., Middleton, WI (U.S. corporation)
PI US 2002010165 A1 20020124
US 6566353 B2 20030520
AI US 2001-891763 A1 20010626 (9)
RLI Continuation-in-part of Ser. No. US 1998-596149, filed on 23 Feb 1998, PENDING Continuation-in-part of Ser. No. US 1996-781910, filed on 30 Dec 1996, GRANTED, Pat. No. US 5763429
DT Utility
FS APPLICATION
LREP MICHAEL BEST & FRIEDRICH, LLP, ONE SOUTH PINCKNEY STREET, P O BOX 1806, MADISON, WI, 53701

CLMN Number of Claims: 33
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1014
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Method of treating malignancy associated hypercalcemia using active **vitamin D** analogues
AB Methods utilizing active **vitamin D** analogs for the treatment of malignancy-associated hypercalcemia. Methods comprise the application of an effective amount of a hypocalcemic **vitamin D** compound to alleviate hypercalcemia, lower serum parathyroid hormone related protein (PTHrP) levels.
SUMM . . . relates generally to a method of treating malignancy-associated hypercalcemia (MAH), and in particular, to the use of active forms of **vitamin D** to reduce hypercalcemia associated with inhibit the hyperproliferative diseases.
SUMM [0004] Extensive research during the past two decades has established important biologic roles for **vitamin D** apart from its classic role in bone and mineral metabolism. Specific nuclear receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3, the hormonally active form of **vitamin D**, are present in cells from diverse organs not involved in calcium homeostasis. For example, specific, biologically active **vitamin D** receptors have been demonstrated in the human prostatic carcinoma cell line, LNCaP, (Miller et al., 52 **Cancer** Res. (1992) 515-520); **Vitamin D** receptors have also been described for many other neoplastic cells, e.g., carcinomas of the breast and carcinomas of the colon.
SUMM [0005] It has been reported that certain **vitamin D** compounds and analogues are potent inhibitors of malignant cell proliferation and are inducers/stimulators of cell differentiation. For example, U.S. Pat.. . . nonmalignant macrophages (monocytes), and are useful in the treatment of leukemia. Antiproliferative and differentiating actions of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues have been reported with respect to **cancer** cell lines. More recently, an association between **vitamin D** receptor gene polymorphism and **cancer** risk has been reported, suggesting that **vitamin D** receptors may have a role in the development, and possible treatment, of **cancer**.
SUMM [0006] These previous studies have focused exclusively on **vitamin D**.sub.3 compounds. Even though these compounds may indeed be highly effective in promoting differentiation in malignant cells in culture, their practical. . . blood calcium levels by virtue of their inherent calcemic activity. That is, the clinical use of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues as anticancer agents is precluded, or severely limited, by the risk of hypercalcemia.
SUMM . . . themselves increase serum calcium levels. Therefore a need exists for compounds with greater specific activity and selectivity of action, i.e., **vitamin D** compounds with antiproliferative and differentiating effects but which have less calcemic activity.
SUMM . . . hypercalcemia (MAH) such as that associated with hyperproliferative cell growth and/or abnormal cell differentiation. The method includes use of active **vitamin D** compounds to treat hypercalcemia and reduce serum parathyroid hormone related protein (PTHrP) levels.
SUMM [0010] A hydroxyvitamin D compound in accordance with the present invention is an active **vitamin D** and is suitably represented by the formula (I) described hereafter. Suitable compounds of formula (I) are 1.alpha.,24-dihydroxyvitamin D.sub.2,

1.alpha.,24-dihydroxyvitamin D.sub.4,. . .

SUMM . . . patients suffering from hypercalcemia is accomplished by a method comprising, administering to these patients an effective amount of a hypocalcemic **vitamin D** compound, to lower the serum parathyroid hormone related protein (PTHrP) level.

SUMM [0013] The hypocalcemic **vitamin D** compounds are also valuable for the treatment of breast, prostate and colon **cancer** , as well as other neoplasms such as pancreatic **cancer**, endometrial **cancer**, testicular **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . tumors, medullary thyroid carcinoma, multiple myeloma, retinoblastoma, and sarcomas of the soft tissue and bone, i.e. neoplasms that express a **vitamin D** receptor.

SUMM [0014] In accordance with the present invention, when effective amounts of the hypocalcemic **vitamin D** compounds are administered to patients with MAH, significantly redeuced hypercalcemia is observed than is observed after the same amount of an activated **vitamin D**.sub.3 (e.g., 1.alpha.-OH D.sub.3, 1.alpha.,25-(OH).sub.2 D.sub.3) is administered in previously known formulations. Thus, the compound in accordance with the present invention has an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues.

SUMM . . . method of treating malignancy associated hyercalcemia comprising administering to a subject who is suffering therefrom an effective amount of active **vitamin D** compound which has, or attains through metabolism in vivo, a **vitamin D** receptor (VDR) binding affinity substantially equivalent to the binding affinity of 1.alpha.,25-dihydroxyvitamin D.sub.3 and has a hypercalcemia risk substantially lower. . .

SUMM [0016] For treatment for malignancy-associated hypercalcemia and the underlying malignant condition in accordance with the present invention, the active **vitamin D** is suitably administered alone as an active ingredient in a pharmaceutical composition, or is co-administered with an anticancer agent.

SUMM [0017] Further, included within the scope of the present invention is the co-administration of a hypocalcemic **vitamin D** compound with a cytotoxic or anticancer agent. Such agents suitably include antimetabolites (e.g., 5-fluoro-uracil, methotrexate, fludarabine), antimicrotubule agents (e.g., vincristine,. . .

SUMM [0018] It is anticipated that the active **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

SUMM [0019] Also included within the scope of the present invention is the co-administration of effective dosages of a hypocalcemic **vitamin D** compound in conjunction with administration of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. For example, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

SUMM [0020] In another aspect, the invention is a pharmaceutical composition which includes an anticancer agent which is an active hypocalcemic **vitamin D** compound; an agent selected from the group consisting of (i) an anticancer agent, (ii) a bone agent, and combinations thereof;. . .

SUMM . . . cells. The present invention provides a novel treatment of a patient suffering from a hyperproliferative disease with an active hypocalcemic **vitamin D** compound. Preferably, the active **vitamin D** analogue is a hydroxyvitamin D compound and is suitably represented by formula (I) as described

hereinbelow. The active **vitamin D** analogue is provided to the patient without itself causing dose-limiting hypercalcemia and hypercalciuria, and in fact, reduces the hypercalcemia caused by the malignancy. These attributes are achieved through specific chemical properties of the hypocalcemic **vitamin D** compounds as described.

SUMM [0024] In accordance with the present invention, when effective amounts of the hypocalcemic active **vitamin D** compounds are administered to patients with malignant diseases, the hypercalcemia is reduced, the PTHrP serum level is reduced, and the. . . of the abnormal cells is inhibited, redeuced, or stabilized, and cell differentiation is induced, promoted or enhanced. Thus, the hypocalcemic **vitamin D** compounds of the present invention have an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues.

SUMM [0025] It is known that **vitamin D**.sub.3 must be hydroxylated in the C-1 and C-25 positions before it is activated, i.e., before it will produce a biological response. A similar metabolism appears to be required to activate other forms of **vitamin D**, e.g., **vitamin D.sub.2** and **vitamin D.sub.4**. Therefore, as used herein, the term "activated **vitamin D**" or "active **vitamin D**" is intended to refer to a **vitamin D** compound or analogue that has been hydroxylated in at least the C-1, C-24 or C-25 position of the molecule and either the compound itself or its metabolites in the case of a prodrug, such as 1.alpha.-hydroxyvitamin D.sub.2, binds the **vitamin D** receptor (VDR). For example, "prodrugs" are **vitamin D** compounds which are hydroxylated in the C-1. Such compounds undergo further hydroxylation in vivo and their metabolites bind the VDR.

SUMM [0026] The term "hypocalcemic **vitamin D** compound" is in reference to active **vitamin D** analogs which demonstrate hypocalcemic activity, i.e. have low calcemic activity relative to that of 1.alpha.,25-dihydroxyvitamin D.sub.3, including 24-hydroxyvitamin D compounds,. . .

SUMM [0029] The compound in accordance with the present invention is an active hypocalcemic **vitamin D** compound. The active **vitamin D** provided is such that the compound has a hydrocarbon moiety at the C-24 position, e.g. a lower alkyl, alkenyl or acyl group as the C-24 position. Further, the active **vitamin D** in accordance with the present invention may have an unsaturated sidechain, e.g., there is suitably a double bond between C-22. . .

SUMM . . . other hyperproliferative cells and can reduce hypercalcemia associated with the maligancy. This selectivity and specificity of action makes the hypocalcemic **vitamin D** compounds useful and preferred antihypercalcemic agents as well as safely inhibiting hyperproliferation and promoting malignant or hyperplastic cell differentiation. The compounds of the present invention, thus, overcome the shortcomings of the known active **vitamin D**.sub.3 compounds described above, and can be considered preferred agents for the control and treatment of malignant diseases such breast, prostate, testicular and colon **cancer**, as well as other neoplasms such as pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . tumors, medullary thyroid carcinoma, multiple myeloma, melanoma retinoblastoma, and sarcomas of the soft tissue and bone, i.e. neoplasms that express **vitamin D** receptors.

SUMM [0037] Suitable hypocalcemic **vitamin D** compounds in accordance with the present invention include: 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin D.sub.2, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.-hydroxyvitamin D.sub.2, and 1.alpha.-hydroxyvitamin. . .

SUMM . . . the present invention provides a method of treating hypercalcemia associated with malignant cells with an effective amount of a hypocalcemic **vitamin D** compound. The effective dosage amount on a daily basis per kilogram of body weight of the patient ranges from about. . .

SUMM . . . the compounds of formula (I) have been studied and compared to that of 1.alpha.,25-dihydroxyvitamin D.sub.3, the active hormonal form of **vitamin D** and the standard against which all **vitamin D** compounds and analogues are measured. For example, it has been found that the **vitamin D** receptor (VDR) binding affinities of the compounds of formula (I), or their active metabolites, are substantially equivalent to (i.e., equal. . .

SUMM . . . At the same time, it has been found that compounds of formula (I) are significantly less toxic than their corresponding **vitamin D**.sub.3 analogues. For example, in parent co-pending application, Ser. No. 08/265,438, the disclosure of which is incorporated herein by reference, the. . .

SUMM [0042] The hypocalcemic **vitamin D** compounds of the present invention are useful as active compounds in pharmaceutical compositions having reduced side effects and low toxicity as compared with the known analogues of active forms of **vitamin D** .sub.3.

SUMM . . . conventional methods of pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans. For example, the hypocalcemic **vitamin D** compounds can be employed in admixtures with conventional excipients, e.g., pharmaceutically acceptable carrier substances suitable for enteral (e.g., oral), parenteral. . .

SUMM . . . wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or one or more other active compounds, for example, **vitamin D**.sub.3 and its 1.alpha.-hydroxylated metabolites, conjugated estrogens or their equivalents, anti-estrogens, calcitonin, biphosphonates, calcium supplements, cobalamin, pertussis toxin and boron.

SUMM . . . 0.01 .mu.g to about 50 .mu.g per gram of composition. For treatment of skin cancers, the dosage of the hypocalcemic **vitamin D** compound in a locally applied composition generally is about 0.01 .mu.g to 100 .mu.g per gram composition.

SUMM [0055] Further, included within the scope of the present invention is the co-administration of a hypocalcemic **vitamin D** compound with a anticancer agent, e.g., a cytotoxic agent. Such agents suitably include antimetabolites (e.g., 5-fluoro-uracil, methotrexate, fludarabine), antimicrotubule agents. . . daunomycin), topoisomerase inhibitors (e.g., etoposide, camptothecins) or any other antineoplastic agents. (estramustine phosphate, prednimustine). It is anticipated that the hypocalcemic **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

SUMM . . . other at a later time, typically within a week. An example of a suitable co-administration regimen is where a hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of a cytotoxic agent.

SUMM . . . of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. As noted above, prostate **cancer** often metastasizes to bone, causing bone loss and

associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

DETD [0061] The affinity of 1.alpha.,24-(OH).sub.2D.sub.2 for the mammalian **vitamin D** receptor (VDR) was assessed using a commercially available kit of bovine thymus VDR and standard 1,25-(OH).sub.2D.sub.3 solutions from Incstar (Stillwater,. . .

DETD [0062] 1.alpha.,24-dihydroxy **vitamin D.sub .4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0063] The VDR affinity binding of 1.alpha.,24-(OH).sub.2D.sub.4 was investigated. The 1.alpha.,24-(OH).sub.2D.sub.4 was incubated with **vitamin D** receptor and radiolabeled tracer 1.alpha.,25-(OH).sub.2D.sub.3. After incubation, the amount of radioactivity bound to the receptor was determined and compared with. .

DETD [0064] These results show that 1.alpha.,24-(OH).sub.2D.sub.4 binds slightly less tightly to the **vitamin D** receptor than does 1.alpha.,25-(OH).sub.2D.sub.3. Such data mean that 1.alpha.,24-(OH).sub.2D.sub.4 has high affinity for the VDR and significant biological activity, similar. . .

DETD . . . results are surprising and unexpected in view of the prior art. They are contrary to the normative wisdom in the **vitamin D** art regarding the very low degree of biological activity of **vitamin D.sub.4** compounds.

DETD [0067] VDR binding of **vitamin D** compounds by prostate cells is demonstrated using the techniques of Skowronski et al., 136 Endocrinology (1995) 20-26, which is incorporated. . .

DETD [0068] 1.alpha.,24-dihydroxy **vitamin D.sub .4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0069] The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,24-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. . .

DETD [0071] The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,25-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. . .

DETD [0072] 1.alpha.,24-dihydroxy **vitamin D.sub .4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0073] Using the plasmids p(CT4).sup.4TKGH, a **vitamin D** receptor (VDR)-expressing plasmid, and pSG5-hVDR1/3, a plasmid containing a Growth Hormone (GH) gene, under the control of a **vitamin D**-responsive element (VDRE), experiments were conducted to explore the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to induce **vitamin D**-dependent growth hormone acting as a reporter gene compared to that of 1.alpha.,25-(OH).sub.2D.sub.3. Cells in culture were transfected with these two plasmids. One plasmid contained the gene for Growth Hormone (GH) under the control of the **vitamin D** responsive element (VDRE) and the other plasmid contained the structural gene for the **vitamin D** receptor (VDR). These transfected cultures were incubated with 1.alpha.,24-(OH).sub.2D.sub.4 or 1.alpha.,25-(OH).sub.2D.sub.3, and the production of growth hormone was measured. Table 2 below shows the results of this assay:

TABLE 2

Induction of Growth Hormone by **Vitamin D** Compounds

Compound	Concentration Used (M)	Growth Hormone Induction (ng/ml)

1,25-(OH).sub.2D.sub.3 1 .times. 10.sup.-10 39
1,25-(OH).sub.2D.sub.3 5 .times. 10.sup.-10 248
1,24-(OH).sub.2D.sub.4 5 .times. 10.sup.-10 165
1,24-(OH).sub.2D.sub.4. . .

DETD [0074] These data show that the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to stimulate **vitamin D**-dependent growth hormone is nearly equivalent to that of 1.alpha.,25-(OH).sub.2D.sub.3. Such results are truly surprising and would not have been expected. . .

DETD [0075] 1.alpha.,24(S)-dihydroxyvitamin D.sub.2 and 1.alpha.,24(R)-dihydroxy-**vitamin D.sub.2**

[1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2]

DETD . . . was conducted to compare the biological activity in vitro of chemically synthesized 1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2, with 1.alpha.,25-(OH).sub.2D.sub.3 and 25-OH-D.sub.3. The **vitamin D**-dependent transcriptional activation model system was used in which plasmids pSG5-hVDR1/3 and p(CT4).sup.4TKGH were co-transfected into Green monkey kidney, COS-1 cells.

DETD [0077] Transfected cells were incubated with **vitamin D** metabolites and growth hormone production was measured. As shown in Table 3, both 1.alpha.,24(S)-(OH).sub.2D.sub.2 and its epimer, 1.alpha.,24(R)-(OH).sub.2D.sub.2, had significantly more activity in this system than 25-OH-D.sub.3, with 1.alpha.,24(S)-(OH).sub.2D.sub.2 having nearly the same activity as 1.alpha.,25-(OH).sub.2D.sub.3.

TABLE 3

Vitamin D-Inducible Growth Hormone Production In Transfected COS-1 Cells

Inducer. . .	Molar Concentra-	Vitamin DCInducible Growth Hormone Production Total GH Production*	Net vitamin DCinducible GH-production

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. Medium containing test analogue or vehicle is replaced every three. . .

DETD [0080] 1.alpha.,24-dihydroxy **vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD [0081] The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD [0083] The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue, 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. After 6-7 days, the medium is removed and stored at. . .

DETD [0088] The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,24-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD [0090] The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,25-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD [0092] Patients with malignancy-associated hypercalcemia participate in an open-label study of a hypocalcemic **vitamin D** compound in accordance with the present invention. Patients are restricted to daily calcium intake of about 400-500 mg. Each patient. . .

CLM What is claimed is:

. . . of treating hypercalcemia associated with malignant or neoplastic cells, comprising treating the cells with an effective amount of a hypocalcemic **vitamin D** compound having a hydrocarbon moiety at the C.sub.24 position.

2. The method of claim 1, wherein the cells are cancers of the breast, colon, **lung**, neck and head, pancreas, endometrium, bladder, cervix, testes, ovaries, squamous cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma,. . .

3. The method of claim 1, wherein the hypocalcemic **vitamin D** is a compound represented by formula (I) ##STR4## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon bond, thus. . .

4. The method of claim 1, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxvitamin D compound is represented by formula (I) ##STR5## wherein A.sup.1 and A.sup.2 each are hydrogen or a. . .

6. A method in accordance with claim 1, wherein a dosing regimen for the hypocalcemic **vitamin D** compound is a daily regimen or an episodic regimen.

8. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is administered daily at a dose of about 10 to 100 .mu.g/day.

9. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is orally, intravenously or regionally delivered to a **cancer** site.

10. A method in accordance with claim 9, wherein the hypocalcemic **vitamin D** compound is administered orally.

11. A method in accordance with claim 1, wherein the hypocalcemic **vitamin D** compound is co-administered with a cytotoxic agent.

22. A method of treating a human to alleviate hypercalcemia associated with breast **cancer**, colon **cancer**, prostate **cancer**, testicular **cancer**, pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . melanoma, retinoblastoma or sarcomas of the soft tissue and bone, comprising administering to the human therapeutic amount of a hypocalcemic **vitamin D** compound.

23. A method of claim 22, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula (III) ##STR6## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon. . .

. . . A method of treating a human to alleviate hypercalcemia associated with malignant cells, comprising administering to the patient a hypocalcemic **vitamin D** compound, and a cytotoxic agent.

27. A method in accordance with claim 26, wherein the hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of the cytotoxic agent.

28. A method in accordance with claim 26, wherein the hypocalcemic **vitamin D** compound is administered 2 to 4 days prior to administration of the cytotoxic agent.

29. A method of claim 26, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula (III) ##STR7## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon. . .

. . . serum parathyroid hormone related protein in a human patient by administering to the human an effective amount of a hypocalcemic **vitamin D** compound.

L4 ANSWER 7 OF 12 USPATFULL
AN 1998:98906 USPATFULL
TI Method of treating prostatic diseases using delayed and/or sustained release **vitamin D** formulations
IN Bishop, Charles W., Madison, WI, United States
Knutson, Joyce C., Madison, WI, United States
Valliere, Charles R., Waunakee, WI, United States
PA Bone Care International, Inc., Madison, WI, United States (U.S. corporation)
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DT Utility
FS Granted
EXNAM Primary Examiner: Killos, Paul J.
LREP Welch, Teresa J.Stroud, Stroud, Willink, Thompson & Howard
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Method of treating prostatic diseases using delayed and/or sustained release **vitamin D** formulations
AB Method of treating prostatic conditions such as prostate **cancer** and hyperplasia by administering 1.alpha.-hydroxyprevitamin D or activated **vitamin D** or a combination thereof in a sustained release form or a delayed and sustained release formulation. Both the sustained release form and the delayed, sustained release form deliver increased active **vitamin D** blood levels without significant risk of hypercalcemia associated with other oral dosing of **vitamin D** forms, to provide the beneficial effect to the diseased prostate tissue.
SUMM . . . hyperproliferative prostatic diseases, and in particular, to the use of delayed and/or sustained release oral medicaments that deliver an active **vitamin D** compound and, more specifically, to delayed and/or sustained release activated

vitamin D or oral 1.alpha.-hydroxyprevitamin D, to inhibit the hyperproliferative cellular activity of these diseases and promote cell differentiation.

SUMM . . . prostate gland gives rise to benign prostatic hyperplasia which is one common prostate disease. Another common prostate disease is prostate **cancer**, especially prostatic adenocarcinoma. Both prostatic hyperplasia and prostate **cancer** have a high rate of incidence in the aging human male population. Approximately one out of every four males above. . .

SUMM Prostate **cancer** is currently the second most frequent cause of **cancer** death after **lung cancer** among American males. Mortality rates for prostate **cancer** increase logarithmically with age and are two times higher in U.S. blacks than whites. Internationally, mortality rates are highest in. . . increase in annual incidence of the disease and a 37% increase in annual mortality rates will be observed. Although prostate **cancer** may be a relatively indolent neoplasm in the elderly, the overall decrease in life span in patients with this disease. . .

SUMM Improvement in the treatment of prostate **cancer** has centered on early detection. In recent years, screening tests which detect certain proteins or peptides secreted by the prostate. . .

SUMM Treatment of prostate **cancer** in men under the age of 65 has focused on radical surgery, e.g., prostatectomy, and/or radiotherapy, but the impact of. . .

SUMM . . . and lumbar vertebrae, causing bone loss and associated pain. Hormone manipulation often may result in significant palliation of metastatic prostate **cancer**, with improvement of bone pain and other disease-associated symptoms. Androgen ablation or control is, thus, also a major adjunctive therapy in advanced metastatic prostate **cancer**.

SUMM In another area of physiology and biochemistry, the **vitamin D** area, extensive research during the past two decades has established important biologic roles for **vitamin D** apart from its classic role in bone and mineral metabolism. Specific nuclear receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3, the hormonally active form of **vitamin D**, are present in cells from diverse organs not involved in calcium homeostasis. For example, Miller et al., 52 **Cancer** Res. (1992) 515-520, have demonstrated biologically active, specific receptors for 1,25-dihydroxyvitamin D.sub.3 in the human prostatic carcinoma cell line, LNCaP.

SUMM It has been reported that certain **vitamin D** compounds and analogues are potent inhibitors of malignant cell proliferation and inducers/stimulators of cell differentiation. 1.alpha.,25-dihydroxyvitamin D.sub.3 has been shown. . . nonmalignant macrophages (monocytes), and are useful in the treatment of leukemia. Antiproliferative and differentiating actions of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues have been reported with respect to prostate **cancer** cell lines. More recently, an association between **vitamin D** receptor gene polymorphism and prostate **cancer** risk has been reported, suggesting that **vitamin D** receptors may have a role in the development, and possible treatment, of prostate **cancer**.

SUMM These previous studies have focused exclusively on **vitamin D**.sub.3 compounds. Even though these compounds may indeed be highly effective in promoting differentiation in malignant cells in culture, their practical. . . blood calcium levels by virtue of their inherent calcemic activity. That is, the clinical use of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues as anti-**cancer** agents is precluded, or severely limited, by the risk of hypercalcemia. This indicates a need

for **vitamin D** therapies with greater specific activity and selectivity of action, i.e., **vitamin D** compounds and/or formulations with antiproliferative and differentiating effects but which have less calcemic activity. In particular, there is a need for **vitamin D** therapies that can be administered orally to provide the active **vitamin D** blood level necessary for antiproliferative and prodifferentiative effects without the risk of hypercalcemia. The need for such **vitamin D** therapies is no greater than in the treatment of prostate hyperplastic and neoplastic prostatic diseases.

SUMM . . . method of treating prostatic disease conditions such as those characterized by hyperproliferative cell growth and/or abnormal cell differentiation, e.g., prostate **cancer** and prostatic hyperplasia. The method includes the administration of a delayed and/or sustained release **vitamin D** therapy to a subject suffering from such diseases to inhibit abnormal cell growth and promote cell differentiation. The delayed and/or sustained release **vitamin D** therapy includes 1.alpha.-hydroxyprevitamin D compounds and/or active **vitamin D** compounds in a delayed and/or sustained release formulation.

SUMM . . . the present invention, are realized in one aspect thereof in a method of inhibiting the hyperproliferative activity of human prostatic **cancer** or hyperplastic cells, comprising treating the cells with an effective amount of a **vitamin D** therapy which is a delayed and/or sustained release **vitamin D** formulation. The treating step includes inhibiting proliferation of, and inducing and enhancing differentiation in such prostatic cells, and the preferred route of administration is oral. The sustained release **vitamin D** formulation includes 1.alpha.-hydroxyprevitamin D and/or an active **vitamin D** in a sustained release matrix. The delayed sustained release formulation further includes an enteric coat of the active ingredient(s).

SUMM . . . D.sub.3, 1.alpha.-hydroxyprevitamin D.sub.3, 1.alpha.,25-dihydroxyprevitamin D.sub.2, 1.alpha.,24-dihydroxyprevitamin D.sub.2, 1.alpha.-hydroxyprevitamin D.sub.2, 1.alpha.,25-dihydroxyprevitamin D.sub.4, 1.alpha.,24-dihydroxyprevitamin D.sub.4, and 1.alpha.-hydroxyprevitamin D.sub.4. Preferred among the active **vitamin D** compounds are 1.alpha.,25-dihydroxyvitamin D.sub.3, 1.alpha.,24-dihydroxyvitamin D.sub.3, 1.alpha.-hydroxyvitamin D.sub.3, 1.alpha.,25-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.-hydroxyvitamin D.sub.2, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.,24-dihydroxyvitamin D.sub.4, and 1.alpha.-hydroxyvitamin. . .

SUMM . . . the 1.alpha.-hydroxyprevitamin D compound, in unit dosage form, is 0.01 .mu.g/kg/day to 2.0 .mu.g/kg/day, and similarly, the amount of active **vitamin D** in delayed and/or sustained release form is 0.01 .mu.g/kg/day to 2.0 .mu.g/kg/day.

SUMM The invention further is a method of treating human prostate **cancer**, comprising administering to a male subject who has prostate **cancer** an effective amount of **vitamin D** compound which compound is 1.alpha.-hydroxyprevitamin D or an active **vitamin D** in delayed and/or sustained release form and which has a hypercalcemia risk substantially lower than that of 1.alpha.,25-dihydroxyvitamin D.sub.3 administered, alone or in previously known formulations, to decrease or stabilize the cellular abnormal proliferative activity of the **cancer**. Thus, in addition to ameliorating prostatic conditions, the formulations of present invention overcome the inherent inadequacies of presently known oral **vitamin D** formulations, by providing a delayed and/or sustained release **vitamin D** oral medicament.

SUMM In one embodiment, the oral medicament is a sustained release (SR) **vitamin D** which includes a 1.alpha.-hydroxyprevitamin

D compound or an active **vitamin D** compound in a sustained release matrix (hereinafter "SR pre D" and "SR active D," respectively). The 1.alpha.-hydroxyprevitamin D is preferably. . .

SUMM . . . the medicament of invention, i.e., 1.alpha.-hydroxyprevitamin D as the active ingredient, 1.alpha.-hydroxyprevitamin D acts as a prodrug for the active **vitamin D** to inhibit abnormal cell proliferation of and induce or enhance cell differentiation in prostatic diseases. The sustained increase in the. . .

SUMM . . . the oral dosage formulation, it is absorbed from the intestine. 1.alpha.-hydroxyprevitamin D is inactive, i.e., does not bind to the **vitamin D** receptor protein and does not stimulate intestinal calcium absorption. As the 1.alpha.-hydroxyprevitamin D is warmed by the core temperature of. . . the animal or human being. Thus, the SR pre D produces a greater sustained blood level of the corresponding activated **vitamin D** with significantly less stimulation of intestinal calcium absorption than is obtained by administering orally the corresponding activated **vitamin D** itself.

SUMM In the SR active D formulation of the present invention, activated **vitamin D** is incorporated in sustained release matrix suitable for oral administration. That is, the activated **vitamin D** is formulated so that it is bound in a matrix which provides a sustained release when exposed to the contents. . .

SUMM In a second embodiment, the oral composition of the present invention is a delayed and sustained release (DSR) **vitamin D,** e.g., a sustained release **vitamin D** with an enteric coating. The 1.alpha.-hydroxyprevitamin D or activated **vitamin D**-containing matrix is suitably covered with an enteric coating that is resistant to disintegration in gastric juices. The enteric coated, sustained release formulation of **vitamin D,** i.e., delayed sustained release **vitamin D,** (hereafter referred to as "DRS pre D" and "DSR activated or active D," respectively) is then administered orally to an. . . the invention travels past the proximal portion of the small intestine, the enteric coating dissolves. The 1.alpha.-hydroxyprevitamin D or active **vitamin D**-containing matrix is exposed to intestinal fluids, and 1.alpha.-hydroxyprevitamin D or activated **vitamin D** is gradually released over a sustained period of time and absorbed from the intestine. Since the major portion of activated **vitamin D** of corresponding 1.alpha.-hydroxyvitamin from the hydroxylated previtamin is absorbed at a point beyond the proximal portion of the small intestine,. . . of hypercalcemia and hypercalciuria, thus increasing the therapeutic window. The gradual release also allows a greater sustained level of activated **vitamin D** compound in the serum to be obtained and, hence, provides a beneficial effect on diseased prostatic tissue.

SUMM The oral DSR composition of present invention may also suitably include a combination of activated previtamin D and activated **vitamin D** (hereafter referred to as "DSR activated pre D and D"). This embodiment of the invention includes one or more of. . .

SUMM Thus, for treatment of prostatic diseases, e.g., prostatic **cancer** or hyperplasia, a subject is provided orally an effective amount of SR **vitamin D** which is 1.alpha.-hydroxyprevitamin D and/or active **vitamin D** in a sustained release matrix, or an effective amount of DSR activated **vitamin D** or DSR activated pre D, or an effective amount of DSR activated pre D and D, thereby increasing the blood level of activated **vitamin D** in an animal or human being, inhibiting prostatic cellular proliferation, and inducing or enhancing cell differentiation.

SUMM For treatment of prostate conditions in accordance with the present invention, SR **vitamin D** or DSR **vitamin**

D is suitably administered alone as an active ingredient (i.e., as a first anticancer agent) or in a mixture including a. . .

SUMM In another aspect, the invention is a pharmaceutical composition which includes a first anticancer agent that is an SR **vitamin D** or a DSR **vitamin D** and an agent selected from the group consisting of (i) a second anticancer agent, (ii) a bone agent, (iii) an androgen ablation agent and (iv) a 5.alpha.-reductase inhibitor and combinations thereof, and a physiologically acceptable carrier. The active **vitamin D** compound is present in a dosage range of about 0.01 .mu.g/kg/day to about 2.0 .mu.g/kg/day; the active previtamin D is. . .

DRWD FIG. 2 is a graph of the expected results of active **vitamin D** concentration versus time after administration of DSR activated D.

DETD . . . relates to therapeutic methods for inhibiting, ameliorating or alleviating the hyperproliferative cellular activity of diseases of the prostate, e.g., prostatic **cancer** and prostatic hyperplasia. The present invention provides a novel treatment for a patient suffering from a hyperproliferative disease such as prostatic **cancer** or prostatic hyperplasia which includes administering a medicament that is 1.alpha.-hydroxyprevitamin D or a DSR active **vitamin D** or active previtamin D. The medicament is provided to the patient without causing dose-limiting hypercalcemia and hypercalciuria, i.e., unphysiologically high. . .

DETD In accordance with the invention, when effective amounts of SR **vitamin D** or DSR **vitamin D** therapies are administered to patients with prostatic **cancer** or prostatic hyperplasia, the proliferative activity of the abnormal prostatic cells is inhibited or alleviated, and cell differentiation is induced or promoted, with significantly less hypercalcemia and hypercalciuria than is observed after the same amount of activated **vitamin D** is administered in previously known formulations. Thus, the medicament of the present invention has an improved therapeutic index. The effective. . . ranges from about 0.01 .mu.g/kg/day to about 2.0 .mu.g/kg/day for 1.alpha.-hydroxyprevitamin D, and 0.01 .mu.g/kg/day to 2.0 .mu.g/kg/day for active **vitamin D**.

DETD It is known that **vitamin D**.sub.3 must be hydroxylated in the C-1 and C-25 positions before it is activated, i.e., before it will produce a biological response. A similar metabolism appears to be required to activate other forms of **vitamin D**, e.g., **vitamin D.sub.2** and **vitamin D.sub.4**. Therefore, as used herein, the term "activated **vitamin D**" or "active **vitamin D**" are intended to refer to a **vitamin D** compound or analogue that has been hydroxylated in at least the C-1 position of the A ring of the molecule and binds or is converted/metabolizes to a compound that binds the **vitamin D** receptor (VDR). In other words, as to the latter, a 1.alpha.-hydroxyvitamin D is further hydroxylated to a compound which is. . .

DETD The 1.alpha.-hydroxyprevitamin D compounds and active **vitamin D** compounds in SR and DSR form of the present invention are those that have effective antiproliferative and cell differentiation activity (i.e., reversal of malignant transformation), particularly with respect to cells of prostatic diseases, e.g., prostatic **cancer** and prostatic hyperplasia, but have a lower tendency or inability to cause the undesired side effects of hypercalcemia and/or hypercalciuria.. . . hyperproliferation and achieving malignant cell differentiation. The formulations of the present invention, thus, overcome the shortcomings of the known active **vitamin D** formulations mentioned above, and can be considered preferred

agents for the control and treatment of malignant diseases such as prostate **cancer** as well as benign prostatic hyperplasia.

DETD . . . embodiment of the medicament of present invention is an SR formulation which includes substantially pure 1.alpha.-hydroxyprevitamin D or an active **vitamin D** contained in a sustained release matrix. It has been found that when substantially pure 1.alpha.-hydroxyprevitamin D is administered orally, it produces a greater sustained increase in the blood level of activated **vitamin D** and significantly less hypercalcemia and hypercalciuria than the same amount of activated **vitamin D** administered orally in previously known formulations. 1.alpha.-Hydroxyprevitamin D is, therefore, useful in the treatment of prostatic conditions. As used herein,. . .

DETD It is known that **vitamin D**.sub.3 is synthesized endogenously in the skin of animals and man from 7-dehydrocholesterol by an ultraviolet-mediated photochemical reaction which breaks the. . . between carbon-4 and carbon-9 to form previtamin D.sub.3. The triene previtamin D.sub.3 is unstable and over time thermally converts to **vitamin D**.sub.3. At normal body temperature an equilibrium exists between previtamin D.sub.3 and **vitamin D**.sub.3, as seen below. ##STR1## As **vitamin D** .sub.3 is further metabolized in vivo this equilibrium shifts to the **vitamin D**.sub.3 form. A similar conversion and equilibrium state exists for 1.alpha.-hydroxyprevitamin D.

DETD . . . cyclic or noncyclic, and wherein the thermal isomer of the 1.alpha.-hydroxyprevitamin D of the general formula (I) is an active **vitamin D** and increases the serum calcium of the **vitamin D**-deficient rat as determined by standard methods used by biochemists in the **vitamin D** area.

DETD . . . is released from the oral dosage formulation, it is absorbed from the intestine. 1.alpha.-Hydroxyprevitamin D does not interact with the **vitamin D** receptor protein of the enterocytes and, therefore, does not stimulate intestinal calcium absorption.

DETD It is also known that the binding of activated **vitamin D** with the **vitamin D** receptor protein of the enterocyte induces the release of enzymes which degrade a significant portion of the unbound activated **vitamin D** present in the intestine. Such degradation decreases the amount of activated **vitamin D** available for absorption into the blood stream. Since 1.alpha.-hydroxyprevitamin D does not bind with the **vitamin D** receptor protein there is no such enzyme induction. Thus, less degradation occurs in the intestine and a greater amount is available for absorption into the blood stream than is the case with the corresponding activated **vitamin D**.

DETD . . . is warmed by the core temperature of the animal or human being, it is thermally converted to the corresponding activated **vitamin D**. The reaction time for thermal conversion to the active form is sufficiently long so that most of the conversion occurs. . . has been absorbed. Thus, the 1.alpha.-hydroxyprevitamin D oral dosage formulation produces a greater sustained blood level of the corresponding activated **vitamin D** with significantly less stimulation of intestinal calcium absorption than is possible with a comparable oral dosage amount of the activated **vitamin D** itself. Thus, oral administration of 1.alpha.-hydroxyprevitamin D provides greater sustained blood levels of active **vitamin D** for treatment of prostatic neoplastic and hyperplastic cells without significant calcemic activity than with comparable oral administration of the active **vitamin D** itself.

DETD The active **vitamin D** of the SR formulation of the medicament of the present invention is preferably a 1.alpha.-hydroxyvitamin D having the general formula. . . 7 carbon atoms, and

can be branched or unbranched, saturated or unsaturated, hetero-substituted or nonhetero-substituted, cyclic or noncyclic, or any **vitamin D** compound or homologue which binds with the **vitamin D** receptor protein.

DETD 1.alpha.-hydroxy-25-fluoro-**vitamin D**.sub.3 [1.alpha.-(OH)-25-FD.sub.3]

DETD 1.alpha.,24-dihydroxy-25-fluoro-**vitamin D**.sub.3 [1.alpha.,24-(OH).sub.2 -25-FD.sub.3];

DETD 1.alpha.-hydroxy-25-fluoro-**vitamin D.sub.2** [1.alpha.-(OH)-25-FD.sub.2]

DETD 1.alpha.,24-dihydroxy-25-fluoro-**vitamin D.sub.2** [1.alpha.,24-(OH).sub.2 -25-FD.sub.2];

DETD 1.alpha.-hydroxy-25-fluoro-**vitamin D.sub.4** [1.alpha.-(OH)-25-FD.sub.4];

DETD 1.alpha.,24-dihydroxy-25-fluoro-**vitamin D.sub.4** [1.alpha.,24-(OH).sub.2 -25-FD.sub.4].

DETD Among those active **vitamin D** compounds that have a chiral center in the side chain, e.g., 1.alpha.,24-dihydroxyvitamin D.sub.2, it is understood that both epimers (e.g.,. . .

DETD The preferred controlled-release oral drug delivery system is the Eudragit RL/RS system in which the active ingredient, activated **vitamin D**, is combined with a sustained release matrix, and sprayed into granules having a dimension of 25/30 mesh. The granules are. . . ingredients will have been slowly but completely released. Accordingly, the ingested tablet will effect a sustained release of the activated **vitamin D** as well as any other active ingredient.

DETD In the second embodiment of the medicament or composition of the present invention, one or more of activated **vitamin D** compounds or one or more substantially pure 1.alpha.-hydroxyprevitamin D or combinations thereof are included in an enteric coated, sustained release. . .

DETD . . . has been found that the DSR activated D formulation of the invention significantly increases the therapeutic window of the activated **vitamin D** compound. That is, the risk of hypercalcemia and hypercalciuria is significantly decreased and the therapeutic effectiveness is significantly increased for the activated **vitamin D** when orally administered in the DSR activated D formulation as compared to the same amount of activated **vitamin D** orally administered in heretofore known oral formulations of those compounds. Furthermore, the DSR activated D formulation permits a higher sustained blood level of the activated **vitamin D** to be obtained than was possible with previously known oral formulations of the activated **vitamin D** compound.

DETD . . . the preferred embodiments are described above, it should be understood that the only limitation as to the kind of active **vitamin D** compound used in this invention is that the **vitamin D** compound itself or its in vivo metabolite binds with the **vitamin D** receptor protein. The only limitation as to the 1.alpha.-hydroxyprevitamin D is that it converts thermally to an active **vitamin D** compound in which it or its in vivo metabolite binds with the VDR.

DETD The compounds of 1.alpha.-hydroxyprevitamin D and active **vitamin D**, preferably of formulas (I), (II),(III) and (IV), are useful as active compounds in the pharmaceutical compositions of the above described. . .

DETD . . . comprising about 0.1 .mu.g/kg/day to about 2.0 .mu.g/kg/day for 1.alpha.-hydroxyprevitamin D and about 0.1 .mu.g/kg/day to 2.0 .mu.g/kg/day for active **vitamin D** with a pharmaceutically acceptable carrier in a suitable matrix and/or enteric coated in accordance with the embodiments of the present. . .

DETD . . . dosage amounts of 0.1 to 2.0 .mu.g per day for 1.alpha.-hydroxyprevitamin D and 0.1 .mu.g to 2.0 .mu.g/day for active **vitamin D**.

DETD . . . within the scope of the present invention is the co-administration of effective dosages of the 1.alpha.-hydroxyprevitamin D or the active **vitamin D** in the DSR or SR formulations of the present invention as first anticancer agents with a second anticancer agent, e.g., a cytotoxic agent, particularly in metastatic prostate **cancer** wherein relapse has occurred following hormonal treatment. Such agents may suitably include estramustine phosphate, prednimustine, cisplatin, 5-fluoro-uracil, melphalan, hydroxyurea, mitomycin, idarubicin, methotrexate, adriamycin and daunomycin. It is anticipated that an active **vitamin D** of formula (III) or (IV) or a 1.alpha.-hydroxyprevitamin D of formula (I) or (II) used in combination with various anticancer. . .

DETD . . . hormones or agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. It is noted above that prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

DETD Male weanling rats are fed a diet deficient in **vitamin D** and with normal calcium (0.47%). After a period of four weeks has elapsed, the rats are divided into two groups,. . .

DETD Male weanling rats are fed a **vitamin D**-deficient diet containing normal Ca (0.47%) and P (0.3%). After four weeks on this diet, the rats are separated into seventeen. . .

DETD Three-week-old rats were maintained on a **vitamin D** -deficient diet containing normal levels of calcium and phosphorus for 3-6 weeks until marked hypocalcemia was observed. The rats then were. .

DETD 1.alpha.,25-(OH).sub.2 preD.sub.3 or 1.alpha.,25-(OH).sub.2 D.sub.3 were incubated with the **vitamin D** receptor protein and tracer amounts of .sup.3 H-1.alpha.,25-(OH).sub.2 D.sub.3 under standard conditions for a competitive binding assay. The amount of. . .

DETD TABLE 4

Binding of 1.alpha.,25-dihydroxyprevitamin D.sub.3 to **Vitamin D** Receptor in vitro

Amount 1,25-preD.sub.3 (pg/tube)	Total Detectable Binding (pg/tube)	Corrected Binding (pg/tube)
7.8	ND	ND
15.6	ND	ND
31.3	ND	ND
62.5	1.88	0.6
125	3.02. . .	

DETD Male weanling rats are fed a **vitamin D**-deficient diet containing normal Ca (0.47%) and P (0.3%). After approximately 4-6 weeks on this diet, the rats are separated into. . .

DETD Bioavailability and Pharmacokinetic Testing of Delayed, Sustained Release Form of Active **Vitamin D** (DSR Active **Vitamin D**)

DETD An appropriate amount of activated **vitamin D** was dissolved in ethanol and combined with the matrix components listed in Table 4 and sprayed onto 850 g 25/30. . .

DETD These results demonstrate that the biological activity of the active **vitamin D** in this DSR formulation is revealed over a sustained period.

DETD An appropriate amount of activated **vitamin D** was

dissolved in ethanol and combined with the matrix components listed in Table 6 and sprayed onto 850 g 25/30. . .

DETD These data show that the active **vitamin D** in this DSR formulation is readily bioavailable.

DETD An appropriate amount of activated **vitamin D** was dissolved in ethanol and combined with the matrix components listed in Table 7 and sprayed onto 850 g 25/30. . .

DETD These data illustrate that the active **vitamin D** in this DSR formulation is readily bioavailable.

DETD . . . 2, 3, 4, 6, 9, 15, 24, 36, and 72 hours after dose administration. The blood is analyzed for active **vitamin D** levels. The animal administered the drug in the capsule formulation shows a slower rise in blood concentration of active **vitamin D**, a lower maximum concentration of active **vitamin D** in the blood and prolonged elevation of active **vitamin D** blood level relative to the animal receiving the drug in fractionated coconut oil.

DETD The graph of FIG. 2 depicts the blood levels of active **vitamin D** expected from the above example.

DETD Delayed and Sustained Levels of Active **Vitamin D** in Serum

DETD Prostate Cell VDR Binding of Active **Vitamin D**

DETD VDR binding of **vitamin D** compounds by prostate cells is demonstrated using the techniques of Skowronski et al., 136 Endocrinology (1995) 20-26, which is incorporated. . .

DETD The procedure of Example 16 is repeated using the active **vitamin D** analogue 1.alpha.,24-dihydroxyvitamin D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,24-(OH).sub.2 D.sub.4 has strong affinity for prostate. . .

DETD The procedure of Example 16 is repeated using the active **vitamin D** analogue 1.alpha.,25-dihydroxyvitamin D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,25-(OH).sub.2 D.sub.4 has strong affinity for prostate. . .

DETD Inhibition of Prostate Cell Proliferation by Active **Vitamin D**

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue, 1.alpha.,24-(OH).sub.2 D.sub.2, at concentrations from 10.sup.-11 to 10.sup.-7 M. Medium containing test analogue or vehicle is replaced every three. . .

DETD The procedure of Example 19 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2 D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,24-(OH).sub.2 D.sub.4 have significantly fewer cells than the. . .

DETD The procedure of Example 19 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2 D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,25-(OH).sub.2 D.sub.4 have significantly fewer cells than the. . .

DETD Stimulation of Prostate Cell Differentiation by Active **Vitamin D**

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue, 1.alpha.,24-(OH).sub.2 D.sub.2, at concentrations from 10.sup.-11 to 10.sup.-7 M. After 6-7 days, the medium is removed and stored at. . .

DETD The procedure of Example 22 is repeated except the active **vitamin D** analogue is 1.alpha.,24-(OH).sub.2 D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,24-(OH).sub.2 D.sub.4 have significantly more PSA than control. . .

DETD The procedure of Example 22 is repeated except the active **vitamin D** analogue is 1.alpha.,25-(OH).sub.2 D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,25-(OH).sub.2

D.sub.4 have significantly more PSA than control. . .

DETD Patients with advanced androgen-independent prostate **cancer** participate in an open-labeled study of 1.alpha.,24-(OH).sub.2 preD.sub.2. Qualified patients are at least 40 years old, exhibit histologic evidence of. . . begin a course of therapy with oral 1.alpha.,24-(OH).sub.2 preD.sub.2 lasting 26 weeks, while discontinuing any previous use of calcium supplements, **vitamin D** supplements, and **vitamin D** hormone replacement therapies. During treatment, the patients are monitored at regular intervals for: (1) hypercalcemia, hyperphosphatemia, hypercalciuria, hyperphosphaturia and other. . .

DETD The study of Example 25 is repeated for the active **vitamin D** compound, 1.alpha.,25-(OH).sub.2 D.sub.2 in DSR form. The results of the phase one study indicate that patients treated with the MTD. . .

DETD In summary, the present invention provides methods for treating prostatic diseases such as prostate **cancer** and prostatic hyperplasia by administration of an oral SR or DSR formulation of 1.alpha.-hydroxyprevitamin D or activated **vitamin D** or combinations thereof. The formulations of the present invention significantly reduce the risk of hypercalcemia and hypercalciuria associated with heretofore known formulations of activated **vitamin D**. Furthermore, the formulation of the invention produces higher levels of activated **vitamin D** for a greater sustained time per administration than is obtained with heretofore known oral formulations of activated **vitamin D**, resulting in improved blood levels of active **vitamin D** reaching the diseased prostate cells.

CLM What is claimed is:

1. A method of inhibiting the hyperproliferative cellular activity of human prostatic **cancer** or hyperplasia, comprising administering to a subject suffering therefrom and having a stomach and a small intestine, an effective amount of an oral medicament including a **vitamin D** compound which is 1.alpha.-hydroxyprevitamin D or an active **vitamin D** contained in a matrix, said matrix having means for releasably binding and controllably releasing said active **vitamin D** over a sustained period of time.

. . . 2. The method of claim 1, wherein said oral medicament further comprises an enteric coating which prevents release of said **vitamin D** compound, said coating being resistant to dissolution in the stomach but predisposed to dissolution in the intestine so as to prevent release of said **vitamin D** compound until said medicament is in the intestine.

. . . but predisposed to dissolution in the middle and distal portion of the intestine so as to prevent release of said **vitamin D** compound until said medicament has traveled to the middle portion of the intestine.

7. The method of claim 1, wherein said active **vitamin D** is 1.alpha.,25-dihydroxyvitamin D.sub.3, 1.alpha.,24-dihydroxyvitamin D.sub.3, 1.alpha.-hydroxyvitamin D.sub.3, 1.alpha.,25-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.-hydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin D.sub.4 or 1.alpha.-hydroxyvitamin D.sub.4.

. . .

. . . in need of such treatment an effective proliferation-inhibiting amount of an oral medicament which is 1.alpha.-hydroxyprevitamin D

compound or a **vitamin D** compound contained in a matrix, said **vitamin D** compound being an active **vitamin D** or a 1.alpha.-hydroxyprevitamin D, said matrix having means for releasably binding and controllably releasing said **vitamin D** compound over a sustained period of time.

10. The method of claim 8, wherein said proliferation-inhibiting amount of active **vitamin D** is 0.01 .mu.g/kg/day to 2.0 .mu.g/kg/day.

11. A method of treating human prostate **cancer**, comprising administering to a male subject who has prostate **cancer** an effective amount of a composition having a first anticancer agent which is 1.alpha.-hydroxyprevitamin D, a sustained release form of an active **vitamin D** compound or a delayed, sustained release form of an active **vitamin D** compound.

15. A pharmaceutical composition, comprising (a) a first anticancer agent which is a **vitamin D** compound selected from the group consisting of a 1.alpha.-hydroxyprevitamin D, an SR active **vitamin D**, DSR active **vitamin D**, and combinations thereof; and (b) an agent selected from the group consisting of (i) a second anticancer agent, (ii) a. . .

16. The pharmaceutical composition of claim 15, wherein said active **vitamin D** compound is selected from the group consisting of 1.alpha.,25-dihydroxyvitamin D.sub.3, 1.alpha.,24-dihydroxyvitamin D.sub.3, 1.alpha.-hydroxyvitamin D.sub.3, 1.alpha.,25-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.-hydroxyvitamin D.sub.2,. . .

19. The pharmaceutical composition of claim 15, wherein said active **vitamin D** compound is present in a dosage range of about 0.01 .mu.g/kg/day to about 2.0 .mu.g/kg/day.

24. A method of treating a human to alleviate the hyperproliferative cellular activity of prostatic **cancer** or hyperplasia, comprising administering to a male human in need thereof a therapeutically effective amount of 1.alpha.-hydroxyprevitamin D or active **vitamin D** in a formulation which is a sustained release form or a delayed, sustained release form, to decrease or stabilize prostate **cancer** or hyperplasia cellular activity and to effect a decreased risk of hypercalcemia.

L4 ANSWER 8 OF 12 USPATFULL
AN 1998:65212 USPATFULL
TI Method of treating prostatic diseases using active **vitamin D** analogues
IN Bishop, Charles W., Madison, WI, United States
Knutson, Joyce C., Madison, WI, United States
Mazess, Richard B., Madison, WI, United States
PA Bone Care International, Inc., Madison, WI, United States (U.S. corporation)
PI US 5763429 19980609
AI US 1996-781910 19961230 (8)
RLI Continuation-in-part of Ser. No. US 1995-415488, filed on 3 Apr 1995, now patented, Pat. No. US 5602116 which is a continuation-in-part of Ser. No. US 1993-119895, filed on 10 Sep 1993, now patented, Pat. No. US 5403831 And a continuation-in-part of Ser. No. US 1995-486387, filed on 7 Jun 1995, now patented, Pat. No. US 5674859 which is a continuation-in-part of Ser. No. US 1994-265438, filed on 24 Jun 1994
DT Utility

FS Granted
EXNAM Primary Examiner: Criares, Theodore J.
LREP Welch, Teresa J.Stroud, Stroud, Willink, Thompson & Howard
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 923
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Method of treating prostatic diseases using active **vitamin D** analogues
AB . . . invention provides therapeutic methods for inhibiting, ameliorating or alleviating the hyperproliferative cellular activity of diseases of the prostate, e.g., prostatic **cancer** and prostatic hyperplasia, which includes administering to a patient in need thereof an active **vitamin D** analogue. Cell differentiation is promoted, induced or enhanced without causing to the patient dose-limiting hypercalcemia and hypercalciuria.
SUMM . . . relates generally to a method of treating hyperproliferative prostatic diseases, and in particular, to the use of active forms of **vitamin D** to inhibit the hyperproliferative cellular activity of these diseases and to promote differentiation of the cells.
SUMM . . . prostate gland gives rise to benign prostatic hyperplasia which is one common prostate disease. Another common prostate disease is prostate **cancer**, especially prostatic adenocarcinoma. Adenocarcinoma of the prostate is common of the fatal pathophysiological prostate cancers, and typically involves a malignant transformation of epithelial cells in the peripheral region of the prostate gland. Both prostatic hyperplasia and prostate **cancer** have a high rate of incidence in the aging human male population. Approximately one out of every four males above. . .
SUMM Prostate **cancer** is currently the second most frequent cause of **cancer** death after **lung cancer** among American males. Mortality rates for prostate **cancer** increase logarithmically with age and are two times higher in U.S. blacks than whites. Internationally, mortality rates are highest in. . . increase in annual incidence of the disease and a 37% increase in annual mortality rates will be observed. Although prostate **cancer** may be a relatively indolent neoplasm in the elderly, the overall decrease in life span in patients with this disease. . .
SUMM Improvement in the treatment of prostate **cancer** has centered on early detection. In recent years, screening tests which detect certain proteins or peptides secreted by the prostate. . .
SUMM Treatment of prostate **cancer** in men under the age of 65 has focused on radical surgery, e.g., prostatectomy, and/or radiotherapy, but the impact of. . .
SUMM . . . and lumbar vertebrae, causing bone loss and associated pain. Hormone manipulation often may result in significant palliation of metastatic prostate **cancer**, with improvement of bone pain and other disease-associated symptoms. Androgen ablation is, thus, also a major adjunctive therapy in advanced metastatic prostate **cancer**.
SUMM . . . unresectable or metastatic disease will eventually fail to respond to further hormonal therapies. A recent study suggests that human prostate **cancer** cells may cycle between being androgen-independent and androgen-dependent. Such cycling may account for the return of the **cancer** after initial improvement. In this large group of patients, other forms of treatment, unfortunately, are far less effective. Radiotherapy often. . .
SUMM In another area of physiology and biochemistry, the **vitamin D** area, extensive research during the past two decades has established important biologic roles for **vitamin D** apart from its classic role in bone and mineral metabolism. Specific

nuclear receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3, the hormonally active form of **vitamin D**, are present in cells from diverse organs not involved in calcium homeostasis. For example, Miller et al., 52 **Cancer** Res. (1992) 515-520, have demonstrated specific, biologically active receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3 in the human prostatic carcinoma cell line, LNCaP.

SUMM It has been reported that certain **vitamin D** compounds and analogues are potent inhibitors of malignant cell proliferation and are inducers/stimulators of cell differentiation. For example, U.S. Pat.. . . nonmalignant macrophages (monocytes), and are useful in the treatment of leukemia. Antiproliferative and differentiating actions of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues have been reported with respect to prostate **cancer** cell lines. More recently, an association between **vitamin D** receptor gene polymorphism and prostate **cancer** risk has been reported, suggesting that **vitamin D** receptors may have a role in the development, and possible treatment, of prostate **cancer**.

SUMM These previous studies have focused exclusively on **vitamin D**.sub.3 compounds. Even though these compounds may indeed be highly effective in promoting differentiation in malignant cells in culture, their practical. . . blood calcium levels by virtue of their inherent calcemic activity. That is, the clinical use of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues as anticancer agents is precluded, or severely limited, by the risk of hypercalcemia. This indicates a need for compounds with greater specific activity and selectivity of action, i.e., **vitamin D** compounds with antiproliferative and differentiating effects but which have less calcemic activity. The need for such compounds is no greater. . .

SUMM . . . method of treating prostatic disease conditions such as those characterized by hyperproliferative cell growth and/or abnormal cell differentiation, e.g., prostate **cancer** and prostatic hyperplasia. The method includes use of active **vitamin D** compounds to inhibit abnormal cell growth and promote cell differentiation.

SUMM The 1.alpha.-hydroxyvitamin D compound is an active **vitamin D** and is suitably represented by the formula (I) described hereinafter. Preferred among the compounds of formula (I), are 1.alpha.,24-dihydroxyvitamin D.sub.2,. . .

SUMM In another aspect, the invention is a method of treating human prostate **cancer**, comprising administering to a male subject who has prostate **cancer** an effective amount of an active **vitamin D** compound which has, or attains through metabolism in vivo, a **vitamin D** receptor (VDR) binding affinity substantially equivalent to the binding affinity of 1.alpha.,25-dihydroxyvitamin D.sub.3 and a hypercalcemia risk substantially lower than that of 1.alpha.,25-dihydroxyvitamin D.sub.3, to decrease or stabilize the cellular abnormal proliferative activity of the **cancer**.

SUMM For treatment for prostate conditions in accordance with the present invention, the active **vitamin D** is suitably administered alone as an active ingredient, i.e., as a first anticancer agent, in a pharmaceutical composition, or in. . .

SUMM In another aspect, the invention is a pharmaceutical composition which includes a first anticancer agent which is an active **vitamin D** compound; an agent selected from the group consisting of (i) a second anticancer agent, (ii) a bone agent, (iii) an. . .

DETD . . . relates to therapeutic methods for inhibiting, ameliorating or alleviating the hyperproliferative cellular activity of diseases of the

prostate, e.g., prostatic **cancer** and prostatic hyperplasia, and inducing, enhancing or promoting cell differentiation in the diseased cells. The present invention provides a novel treatment of a patient suffering from a hyperproliferative disease such as prostatic **cancer** or prostatic hyperplasia with an active **vitamin D** analogue having a hydrocarbon moiety substituted at the C-24 position of the sidechain of the molecule. Preferably, the active **vitamin D** analogue is a 1.alpha.-hydroxyvitamin D compound and is suitably represented by formula (I) as described hereinbelow. The active **vitamin D** analogue is provided to the patient without causing dose-limiting hypercalcemia and hypercalciuria, i.e., unphysiologically high and deleterious blood calcium levels. . .

DETD . . . accordance with the present invention, when effective amounts of the analogues of formula (I) are administered to patients with prostatic **cancer** or prostatic hyperplasia, the proliferative activity of the abnormal prostatic cells is inhibited or alleviated, and cell differentiation is induced, promoted or enhanced, with significantly less hypercalcemia and hypercalciuria than is observed after the same amount of activated **vitamin D**.sub.3 is administered in previously known formulations. Thus, the compounds of formula (I) have an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues.

DETD It is known that **vitamin D**.sub.3 must be hydroxylated in the C-1 and C-25 positions before it is activated, i.e., before it will produce a biological response. A similar metabolism appears to be required to activate other forms of **vitamin D**, e.g., **vitamin D.sub.2** and **vitamin D.sub.4**. Therefore, as used herein, the term "activated **vitamin D**" or "active **vitamin D**" is intended to refer to a **vitamin D** compound or analogue that has been hydroxylated in at least the C-1 position of the A ring of the molecule. . . and either the compound itself or its metabolites in the case of a prodrug, such as 1.alpha.-hydroxyvitamin D.sub.2, binds the **vitamin D** receptor (VDR). **Vitamin D** compounds which are hydroxylated only in the C-1 position are referred to herein as "prodrugs." Such compounds undergo further hydroxylation. . .

DETD The compound in accordance with the present invention is an active **vitamin D** compound provided that such compound has a hydrocarbon moiety at the C-24 position, e.g., a lower alkyl, alkenyl or acyl group at the C-24 position. Further, the active **vitamin D** in accordance with the present invention may have an unsaturated sidechain, e.g., there is suitably a double bond between C-22. . .

DETD . . . antiproliferative and cell differentiation activity (i.e., reversal of malignant transformation), particularly with respect to cells of prostatic diseases, e.g., prostatic **cancer** and prostatic hyperplasia, but have a lower tendency or inability to cause the undesired side effects of hypercalcemia and/or hypercalciuria.. . . or hyperplastic cell differentiation. The 1.alpha.-hydroxyvitamin D compounds of the present invention, thus, overcome the shortcomings of the known active **vitamin D**.sub.3 compounds described above, and can be considered preferred agents for the control and treatment of malignant diseases such as prostate **cancer** as well as benign prostatic hyperplasia.

DETD Preferred among the active **vitamin D** compounds of formula (I) are: 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha., 25-dihydroxyvitamin D.sub.2, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.-hydroxyvitamin D.sub.2, and 1.alpha.-hydroxyvitamin D.sub.4. Among. . .

DETD The compounds of formula (I) are valuable for the treatment of prostate **cancer** and prostatic hyperplasia in a patient suffering therefrom. In particular, the invention is a method for treating a patient suffering from the hyperproliferative cellular effects of prostate **cancer** and prostatic hyperplasia by administering to the patient a therapeutically effective amount of a compound of formula (I), which is. . .

DETD . . . the compounds of formula (I) have been studied and compared to that of 1.alpha.,25-dihydroxyvitamin D.sub.3, the active hormonal form of **vitamin D** and the standard against which all **vitamin D** compounds and analogues are measured. For example, it has been found that the **vitamin D** receptor (VDR) binding affinities of the compounds of formula (I), or their active metabolites, are substantially equivalent to (i.e., equal. . .

DETD At the same time, it has been found that compounds of formula (I) are significantly less toxic than their corresponding **vitamin D**.sub.3 analogues. For example, in parent co-pending application, Ser. No. 08/265,438, the disclosure of which is incorporated herein by reference, the. . .

DETD . . . in pharmaceutical compositions having reduced side effects and low toxicity as compared with the known analogues of active forms of **vitamin D**.sub.3.

DETD . . . of the pharmaceutical compositions of the present invention is preferred. The dosage of the compounds for the treatment of prostatic **cancer** or hyperplasia according to this invention generally is about 0.01 to about 2.0 .mu.g/kg/day, preferably about 0.01 to about 1.0. . .

DETD For treatment of prostate **cancer**, the parenteral dosage of the compounds of formula (I) is about 0.01 .mu.g/kg/day to about 1.0 .mu.g/kg/day.

DETD Further, included within the scope of the present invention is the co-administration of the active **vitamin D** of formula (I) with a second anticancer agent, e.g., a cytotoxic agent, particularly in metastatic prostate **cancer** wherein relapse has occurred following hormonal treatment. Such agents may suitably include estramustine phosphate, prednimustine, cisplatin, 5-fluoro-uracil, melphalan, hydroxyurea, mitomycin, idarubicin, methotrexate, adriamycin and daunomycin. It is anticipated that an active **vitamin D** of formula (I) used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on. . .

DETD . . . of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. As noted above, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

DETD The affinity of 1.alpha.,24-(OH).sub.2 D.sub.2 for the mammalian **vitamin D** receptor (VDR) was assessed using a commercially available kit of bovine thymus VDR and standard 1,25-(OH).sub.2 D.sub.3 solutions from Incstar. . .

DETD Example 2: 1.alpha.,24-dihydroxy **vitamin D**. **sub.4** [1.alpha.,24-(OH).sub.2 D.sub.4]

DETD The VDR affinity binding of 1.alpha.,24-(OH).sub.2 D.sub.4 was investigated. The 1.alpha.,24-(OH).sub.2 D.sub.4 was incubated with **vitamin D** receptor and radiolabeled tracer 1.alpha.,25-(OH).sub.2 D.sub.3. After incubation, the amount of radioactivity bound to the receptor was determined and compared. . .

DETD These results show that 1.alpha.,24-(OH).sub.2 D.sub.4 binds slightly less tightly to the **vitamin D** receptor than does 1.alpha.,25-(OH).sub.2 D.sub.3. Such data mean that 1.alpha.,24-(OH).sub.2 D.sub.4 has high affinity for the VDR and significant

biological. . .

DETD . . . results are surprising and unexpected in view of the prior art. They are contrary to the normative wisdom in the **vitamin D** art regarding the very low degree of biological activity of **vitamin D.sub.4** compounds.

DETD VDR binding of **vitamin D** compounds by prostate cells is demonstrated using the techniques of Skowronski et al., 136 Endocrinology (1995) 20-26, which is incorporated. . .

DETD Example 4: 1.alpha.,24-dihydroxy **vitamin D.sub.4** [1.alpha.,24-(OH).sub.2 D.sub.4]

DETD The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2 D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,24-(OH).sub.2 D.sub.4 has strong affinity for prostate. . .

DETD The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2 D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,25-(OH)2D.sub.4 has strong affinity for prostate VDR,. . .

DETD Example 6: 1.alpha.,24-dihydroxy **vitamin D.sub.4** [1.alpha.,24-(OH).sub.2 D.sub.4]

DETD Using the plasmids p(CT4).sup.4 TKGH, a **vitamin D** receptor (VDR)-expressing plasmid, and pSG5-hVDR1/3, a plasmid containing a Growth Hormone (GH) gene, under the control of a **vitamin D**-responsive element (VDRE), experiments were conducted to explore the ability of 1.alpha.,24-(OH).sub.2 D.sub.4 to induce **vitamin D**-dependent growth hormone acting as a reporter gene compared to that of 1.alpha.,25-(OH).sub.2 D.sub.3. Cells in culture were transfected with these two plasmids. One plasmid contained the gene for Growth Hormone (GH) under the control of the **vitamin D** responsive element (VDRE) and the other plasmid contained the structural gene for the **vitamin D** receptor (VDR). These transfected cultures were incubated with 1.alpha.,24-(OH).sub.2 D.sub.4 or 1.alpha.,25-(OH).sub.2 D.sub.3, and the production of growth hormone was. . .

DETD TABLE 2

Induction of Growth Hormone by **Vitamin D** Compounds

Compound	Concentration Used (M)	Growth Hormone Induction (ng/ml)
1,25-(OH).sub.2 D.sub.3	1 .times. 10.sup.-10	39
1,25-(OH).sub.2 D.sub.3	5 .times. 10.sup.-10	248
1,24-(OH).sub.2 D.sub.4	5. . .	

DETD These data show that the ability of 1.alpha.,24-(OH).sub.2 D.sub.4 to stimulate **vitamin D**-dependent growth hormone is nearly equivalent to that of 1.alpha.,25-(OH).sub.2 D.sub.3. Such results are truly surprising and would not have been. . .

DETD . . . compare the biological activity in vitro of chemically synthesized 1.alpha.,24(S)-(OH).sub.2 D.sub.2 and 1.alpha.,24(R)-(OH).sub.2 D.sub.2, with 1.alpha.,25-(OH).sub.2 D.sub.3 and 25-OH-D.sub.3. The **vitamin D**-dependent transcriptional activation model system was used in which plasmids pSG5-hVDR1/3 and p(CT4).sup.4 TKGH were co-transfected into Green monkey kidney, COS-1. . .

DETD Transfected cells were incubated with **vitamin D** metabolites and growth hormone production was measured. As shown in

Table 3, both 1.alpha.,24(S)-(OH).sub.2 D.sub.2 and its epimer, 1.alpha.,24(R)-(OH).sub.2 D.sub.2,. . .

DETD TABLE 3

Vitamin D-Inducible Growth Hormone Production In Transfected COS-1 Cells

Inducer	Molar Concentration	**Vitamin D**-Inducible Growth Hormone Production: Total GH Production (ng/ml)	**Vitamin D**-Inducible Growth Hormone Production: Net **vitamin D**-inducible GH-production* (ng/ml)
Ethanol		44	0
25-OH-D.sub.3	1 .times. 10.sup.-7	245	201
	1 .times. 10.sup.-6	1100	1056
	1 .times.. . .		

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2 D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. Medium containing test analogue or vehicle is replaced every. . .

DETD Example 9: 1.alpha.,24-dihydroxy **vitamin D**. **sub.4** [1.alpha.,24-(OH).sub.2 D.sub.4]

DETD The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2 D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,24-(OH).sub.2 D.sub.4 have significantly fewer cells than the. . .

DETD The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2 D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,25-(OH).sub.2 D.sub.4 have significantly fewer cells than the. . .

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue, 1.alpha.,24-(OH).sub.2 D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. After 6-7 days, the medium is removed and stored. . .

DETD The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,24-(OH).sub.2 D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,24-(OH).sub.2 D.sub.4 have significantly more PSA than control. . .

DETD The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,25-(OH).sub.2 D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,25-(OH).sub.2 D.sub.4 have significantly more PSA than control. . .

DETD Example 14: 1.alpha.,24-dihydroxy **vitamin D**. **sub.2** [1.alpha.,24-(OH).sub.2 D.sub.2]

DETD Patients with advanced androgen-independent prostate **cancer** participate in an open-labeled study of 1.alpha.,24-(OH).sub.2 D.sub.2. Qualified patients are at least 40 years old, exhibit histologic evidence of. . . begin a course of therapy with oral 1.alpha.,24-(OH).sub.2 D.sub.2 lasting 26 weeks, while discontinuing any previous use of calcium supplements, **vitamin D** supplements, and **vitamin D** hormone replacement therapies. During treatment, the patients are monitored at regular intervals for: (1) hypercalcemia, hyperphosphatemia, hypercalciuria, hyperphosphaturia and other. . .

DETD The study of Example 14 is repeated for the active **vitamin D** compound, 1.alpha.-OH-D.sub.2. The results of the phase one study indicate that patients treated with the MTD of 1.alpha.-OH-D.sub.2 for at. . .

CLM What is claimed is:

7. A method of treating human prostate **cancer**, comprising administering to a male subject, who has prostate **cancer**, an effective amount of a first anticancer agent which is an active **vitamin-D** compound to decrease or stabilize the cellular abnormal proliferative activity of the **cancer**, said compound or its in vivo metabolite having a VDR binding affinity substantially equivalent to the binding affinity of 1.alpha.,25-dihydroxyvitamin. . .

8. The method of claim 7, wherein said active **vitamin D** is administered in a mixture including a second anticancer agent selected from the group consisting of estramustine phosphate, prednimustine, cisplatin,. . .

9. A method of treating a human to alleviate the hyperproliferative cellular activity of prostatic **cancer** or hyperplasia, comprising administering to a human in need thereof a therapeutically effective amount of an active **vitamin D** compound having a hydrocarbon moiety substituted at C-24.

L4 ANSWER 9 OF 12 USPATFULL
AN 86:60714 USPATFULL
TI Utilization of a single vitamin or a combination of various vitamins
IN Motschan, Georges, Schonbeinstrasse 21, 4056 Basel, Switzerland
PI US 4619829 19861028
WO 8401899 19840524
AI US 1984-631555 19840713 (6)
WO 1983-CH127 19831116
19840713 PCT 371 date
19840713 PCT 102(e) date
PRAI CH 1982-6682 19821116
DT Utility
FS Granted
EXNAM Primary Examiner: Robinson, Douglas W.
LREP Ostrolenk, Faber, Gerb & Soffen
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 637
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

SUMM **VITAMIN D**

SUMM . . . of related compounds have been detected, all of which together with the former named vitamins comprise the so-called group of **vitamin-D**.

SUMM **Vitamin D**.sub.1 : Molecular compound of **Vitamin D.sub.2** and lumisterol.sub.2 (irradiated product of ergosterol). None-existant in nature.

SUMM **Vitamin D.sub.4** : Irradiation product of 22,23-dihydroergosterol;

SUMM **Vitamin D**.sub.5 : Irradiation product of 7-dehydrositosterol;

SUMM **Vitamin D**.sub.6 : Irradiation product of 7-dehydrostigmasterol;

SUMM **Vitamin D**.sub.7 : Irradiation product of 7-dehydrocamposterol (isomer to 22,23-dihydroergosterol).

SUMM . . . B.sub.12
5 .mu.g 5 .mu.g

					5	.mu.g
Folic acid	1	mg	1	mg	1	mg
Vitamin C	150	mg	150	mg	150	mg
Vitamin D	500	I.U.	500	I.U.	1000	I.U.
Vitamin E	10	mg	10	mg	10	mg

DETD Father of person B died at the age of 59 (1948) of **lung cancer**. Both elder sisters of person B died at the ages of 45 and 58, both of carzinoma of the breast.

CLM What is claimed is:

. . . Panthenol 0.25 mg Biotin (Vitamin H) 5 ug Vitamin B.sub.12 1 mg Folic Acid 150 mg Vitamin C 1,000 I.U. **Vitamin D** 10 mg Vitamin E, 435 mg CaHPO.sub.4.3H.sub.2 O 10 mg Fe reduct, 36.5 mg MgHPO.sub.4.3H.sub.2 O 2.05 mg MnSO.sub.4.4H.sub.2 O. . .

L4 ANSWER 10 OF 12 USPAT2

AN 2002:43583 USPAT2

TI Method of treating hyperproliferative diseases using active **vitamin D** analogues

IN Bishop, Charles W., Madison, WI, United States
Mazess, Richard B., Madison, WI, United States

PA Bone Care International, Inc., Middleton, WI, United States (U.S. corporation)

PI US 6503893 B2 20030107

AI US 2001-891814 20010626 (9)

RLI Continuation-in-part of Ser. No. US 1998-596149, filed on 23 Feb 1998 Division of Ser. No. US 1996-781910, filed on 30 Dec 1996, now patented, Pat. No. US 5763429

DT Utility

FS GRANTED

EXNAM Primary Examiner: Reamer, James H

LREP Michael, Best & Friedrich LLP, Welch, Teresa J., Peterson, Jeffrey D.

CLMN Number of Claims: 44

ECL Exemplary Claim: 1

DRWN 0 Drawing Figure(s); 0 Drawing Page(s)

LN.CNT 1227

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

TI Method of treating hyperproliferative diseases using active **vitamin D** analogues

AB Methods for the utilization of hypocalcemic **vitamin D** analogs to inhibit the hyperproliferation of malignant or neoplastic cells without incidence of hypercalcemia.

SUMM . . . relates generally to a method of treating hyperproliferative diseases, and in particular, to the use of active forms of hypocalcemic **vitamin D** to inhibit the hyperproliferative cellular activity of these diseases and to promote differentiation of the cells.

SUMM Extensive research during the past two decades has established important biologic roles for **vitamin D** apart from its classic role in bone and mineral metabolism. Specific nuclear receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3, the hormonally active form of **vitamin D**, are present in cells from diverse organs not involved in calcium homeostasis. For example, specific, biologically active **vitamin D** receptors have been demonstrated in the human prostatic carcinoma cell line, LNCaP, (Miller et al., 52 **Cancer** Res. (1992) 515-520); **Vitamin D** receptors have also been described for many other neoplastic cells, e.g., carcinomas of the breast and the colon.

SUMM It has been reported that certain **vitamin D** compounds and analogues are potent inhibitors of malignant cell proliferation and are inducers/stimulators of cell differentiation. For example, U.S. Pat.. . . nonmalignant macrophages (monocytes), and are useful in the treatment of leukemia. Antiproliferative and

differentiating actions of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin** D.sub.3 analogues have been reported with respect to **cancer** cell lines. More recently, an association between **vitamin D** receptor gene polymorphism and **cancer** risk has been reported, suggesting that **vitamin D** receptors may have a role in the development, and possible treatment, of **cancer**.

SUMM These previous studies have focused exclusively on **vitamin** D.sub.3 compounds. Even though these compounds may indeed be highly effective in promoting differentiation in malignant cells in culture, their practical. . . blood calcium levels by virtue of their inherent calcemic activity. That is, the clinical use of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin** D.sub.3 analogues as anticancer agents is precluded, or severely limited, by the risk of hypercalcemia. This indicates a need for compounds with greater specific activity and selectivity of action, i.e., **vitamin D** compounds with antiproliferative and differentiating effects but which have less calcemic activity.

SUMM . . . disease conditions such as those characterized by hyperproliferative cell growth and/or abnormal cell differentiation. The method includes use of active **vitamin D** compounds to inhibit abnormal cell growth and promote cell differentiation.

SUMM . . . inhibiting the hyperproliferative activity of neoplastic or hyperplastic cells, comprising treating the cells with an effective amount of a hypocalcemic **vitamin D** compound. The treating step includes inhibiting proliferation of, and inducing and enhancing differentiation in such cells.

SUMM The hypocalcemic **vitamin D** compounds of the present invention include **vitamin D** compounds having a hydrocarbon moiety substituted at the C-24 position on the sidechain of the molecule and a hydroxy group. . .

SUMM The **vitamin D** compound of the present invention is an active **vitamin D** and is suitably represented by the formula (I) described hereafter. The compounds of formula (I) suitably include 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin. . .

SUMM Hypocalcemic **vitamin D** compounds are valuable for the treatment of breast and colon **cancer**, as well as other neoplasms such as pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell **cancer** of the head and neck, bladder, ovarian and cervical cancers, myeloid and lymphocyltic leukemia, lymphoma, hepatic tumors, medullary thyroid carcinoma,. . .

SUMM In accordance with the present invention, when effective amounts of hypocalcemic **vitamin D** compounds are administered to patients with **cancer** or neoplasms, the proliferative activity of the abnormal neoplastic cells is inhibited, reduced, or stabilized, and cell differentiation is induced, promoted or enhanced, with significantly less hypercalcemia and hypercalciuria than is observed after the same amount of an activated **vitamin D** .sub.3 (e.g., 1.alpha.-OH D.sub.3, 1.alpha.,25-(OH).sub.2 D.sub.3) is administered in previously known formulations. Thus, the compound in accordance with the present invention has an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues.

SUMM Accordingly, another aspect of the invention is a method of treating human **cancer** comprising administering to a subject who has **cancer** an effective amount of hypocalcemic **vitamin D** compound which has or attains through metabolism in vivo, a **vitamin D** receptor (VDR) binding affinity substantially equivalent to the binding affinity of 1.alpha.,25-

dihydroxyvitamin D.sub.3 and a hypercalcemia risk substantially lower that that of 1.alpha.,25-dihydroxyvitamin D.sub.3, to inhibit, decrease or stabilize the cellular abnormal proliferative activity of the **cancer**.

SUMM For treatment for malignant conditions in accordance with the present invention, the hypocalcemic **vitamin D** compounds can be suitably administered alone as an active ingredient, as an antiproliferative agent in a pharmaceutical composition, or co-administered. . .

SUMM Further, included within the scope of the present invention is the co-administration of the **vitamin D** of formula (I) with a cytotoxic or anticancer agent. Such agents suitably include antimetabolites (e.g., 5-fluoro-uracil, methotrexate, fludarabine), antimicrotubule agents. . .

SUMM It is anticipated that the hypocalcemic **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

SUMM . . . administration of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. For example, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

SUMM In another aspect, the invention is a pharmaceutical composition which includes an anticancer agent which is an active **vitamin D** compound; an agent selected from the group consisting of (i) an anticancer agent, (ii) a bone agent, and combinations thereof;. . .

DETD . . . diseased cells. The present invention provides a novel treatment of a patient suffering from a hyperproliferative disease such as prostatic **cancer** or prostatic hyperplasia with a hypocalcemic hydroxyvitamin D analogue. The **vitamin D** analogue is suitably a 1.alpha.-hydroxyvitamin D or a 24-hydroxyvitamin D compound. The hypocalcemic hydroxyvitamin D analogue represented by formula (I). . . deleterious blood calcium levels and urine calcium levels, respectively. These attributes are achieved through specific chemical properties of the hypocalcemic **vitamin D** compounds as described.

DETD In accordance with the present invention, when effective amounts of the hypocalcemic **vitamin D** compounds are administered to patients with **cancer** or hyperplasia, the proliferative activity of the abnormal cells is inhibited, maintained, or alleviated, and cell differentiation is induced, promoted or enhanced, with significantly less hypercalcemia and hypercalciuria than is observed after the same amount of activated **vitamin D**.sub.3 is administered in previously known formulations. Thus, the hypocalcemic **vitamin D** compounds of the present invention have an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues.

DETD It is known that **vitamin D**.sub.3 must be hydroxylated in the C-1 and C-25 positions before it is activated, i.e., before it will produce a biological response. A similar metabolism appears to be required to activate other forms of **vitamin D**, e.g., **vitamin D.sub.2** and **vitamin D.sub.4**. Therefore, as used herein, the term "activated **vitamin D**" or "active **vitamin D**" is intended to refer to a **vitamin D** compound or analogue that has been hydroxylated in at least the C-1, C-24 or C-25 position of the molecule and either the compound itself or its metabolites in the case of a prodrug, such as 1.alpha.-hydroxyvitamin D.sub.2, binds the **vitamin D** receptor (VDR). For example, **vitamin D** "prodrugs" include compounds which are hydroxylated in the C-1

position. Such compounds undergo further hydroxylation in vivo and their metabolites. . .

DETD The term "hypocalcemic **vitamin D** compound" is in reference to active **vitamin D** analogs which demonstrate reduced calcemic activity relative to the calcemic activity of 1.alpha.,25-dihydroxyvitamin D.sub.3. Such compounds include 24-hydroxyvitamin D compounds,. . .

DETD The compound in accordance with the present invention is an active hypocalcemic **vitamin D** compound. Further, the active **vitamin D** in accordance with the present invention may have an unsaturated sidechain, e.g., there is suitably a double bond between C-22. . .

DETD The hypocalcemic **vitamin D** compounds of the present invention are those that have effective antiproliferative and cell differentiation activity (i.e., reversal of malignant transformation),. . . to malignant or other hyperproliferative cells without significantly altering calcium metabolism. This selectivity and specificity of action makes the hypocalcemic **vitamin D** compounds useful and preferred agents for safely inhibiting hyperproliferation and promoting malignant or hyperplastic cell differentiation. The compounds of the present invention, thus, overcome the shortcomings of the known active **vitamin D**.sub.3 compounds described above, and can be considered preferred agents for the control and treatment of malignant diseases such breast, prostate, testicular and colon **cancer**, as well as other neoplasms such as pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . medullary thyroid carcinoma, multiple myeloma, melanoma, retinoblastoma, and sarcomas of the soft tissue and bone, i.e. neoplasms that express a **vitamin D** receptor.

DETD . . . hyperproliferative cells, (i.e., inhibiting their hyperproliferative activity and/or inducing and enhancing their differentiation) with an effective amount of a hypocalcemic **vitamin D** compound. The effective dosage amount on a daily basis per kilogram of body weight of the patient ranges from about. . . dose is given. The compounds in accordance with the present invention are administered in an amount that raises a serum **vitamin D** level to a supraphysiological level for a sufficient period of time to induce differentiation or regression of a tumor or. . .

DETD The compounds of formula (I) are valuable for the treatment of **cancer** and neoplasms in a patient suffering therefrom. In particular, the invention is a method for treating a patient suffering from the hyperproliferative cellular effects of **cancer** and other neoplasms by administering to the patient a therapeutically effective amount of a compound of formula (I), which is. . .

DETD . . . of the compounds of formula(I) have been studied and compared to that of 1.alpha.,25-dihydroxyvitamin D.sub.3, the active hormonal form of **vitamin D** and the standard against which all **vitamin D** compounds and analogues are measured. For example, it has been found that the **vitamin D** receptor (VDR) binding affinities of the compounds of formula (I), or their active metabolites, are substantially equivalent to (i.e., equal. . .

DETD At the same time, it has been found that compounds of formula (I) are significantly less toxic than their corresponding **vitamin D**.sub.3 analogues. For example, in parent co-pending application, Ser. No. 08/265,438, the disclosure of which is incorporated herein by reference, the. . .

DETD . . . in pharmaceutical compositions having reduced side effects and

low toxicity as compared with the known analogues of active forms of **vitamin D**.sub.3.

DETD . . . conventional methods of pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans. For example, the hypercalcemic **vitamin D** compounds of the present invention can be employed in admixtures with conventional excipients, e.g., pharmaceutically acceptable carrier substances suitable for. . .

DETD . . . wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or one or more other active compounds, for example, **vitamin D**.sub.3 and its 1.alpha.-hydroxylated metabolites, conjugated estrogens or their equivalents, anti-estrogens, calcitonin, biphosphonates, calcium supplements, cobalamin, pertussis toxin and boron.

DETD . . . about 0.01 .mu.g to about 50 .mu.g per gram of composition. For treatment of cancers, the dosage of the hypocalcemic **vitamin D** compound in a locally applied composition generally is about 0.01 .mu.g to 100 .mu.g per gram composition.

DETD . . . administration of the pharmaceutical compositions of the present invention is preferred. The dosage of the compounds for the treatment of **cancer** or neoplasms according to this invention generally is about 0.01 to about 2.0 .mu.g/kg/day, preferably about 0.01 to about 1.0 .mu.g/kg/day. As noted above, dosing of the hypocalcemic **vitamin D** compounds in accordance with the present invention can be done on an episodic basis, in which higher does can be. . .

DETD Further, included within the scope of the present invention is a method of co-administration of hypercalemic **vitamin D** compounds with an anticancer or antineoplastic agent. Such agents may suitably include antimetabolites (e.g., 5-fluorouracil, methotrexate, fludarabine), antimicrotubule agents (e.g.,. . . adriamycin, daunomycin), topoisomerase inhibitiors (e.g., etoposide, camptothecins) or any other antineoplastic agents. (estramustine phosphate, prednimustine). It is anticipated that hypercalcemic **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

DETD . . . other at a later time, typically within a week. An example of a suitable co-administration regimen is where a hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of a cytotoxic agent.

DETD Also included within the scope of the present invention is the co-administration of effective dosages of hypercalcemic **vitamin D** compounds in conjunction with administration of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. For example, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

DETD The affinity of 1.alpha.,24-(OH).sub.2D.sub.2 for the mammalian **vitamin D** receptor (VDR) was assessed using a commercially available kit of bovine thymus VDR and standard 1,25-(OH).sub.2D.sub.3 solutions from Incstar (Stillwater,. . .

DETD 1.alpha.,24-Dihydroxy **Vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD The VDR affinity binding of 1.alpha.,24-(OH).sub.2D.sub.4 was investigated. The 1.alpha.,24-(OH).sub.2D.sub.4 was incubated with **vitamin D** receptor and radiolabeled tracer 1.alpha.,25-(OH).sub.2D.sub.3. After incubation, the amount of radioactivity bound to the receptor was determined and compared with. .

DETD These results show that 1.alpha.,24-(OH).sub.2D.sub.4 binds slightly less tightly to the **vitamin D** receptor than does

1.alpha.,25-(OH).sub.2D.sub.3. Such data mean that 1.alpha.,24-(OH).sub.2D.sub.4 has high affinity for the VDR and significant biological activity, similar. . .

DETD . . . results are surprising and unexpected in view of the prior art. They are contrary to the normative wisdom in the **vitamin D** art regarding the very low degree of biological activity of **vitamin D.sub.4** compounds.

DETD VDR binding of **vitamin D** compounds by prostate cells is demonstrated using the techniques of Skowronski et al., 136 Endocrinology (1995) 20-26, which is incorporated. . .

DETD 1.alpha.,24-Dihydroxy **Vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,24-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. .

.

DETD The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,25-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. .

.

DETD Dihydroxy **Vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD Using the plasmids p(CT4).sup.4TKGH, a **vitamin D** receptor (VDR)-expressing plasmid, and pSG5-hVDR1/3, a plasmid containing a Growth Hormone (GH) gene, under the control of a **vitamin D**-responsive element (VDRE), experiments were conducted to explore the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to induce **vitamin D**-dependent growth hormone acting as a reporter gene compared to that of 1.alpha.,25-(OH).sub.2D.sub.3. Cells in culture were transfected with these two plasmids. One plasmid contained the gene for Growth Hormone (GH) under the control of the **vitamin D** responsive element (VDRE) and the other plasmid contained the structural gene for the **vitamin D** receptor (VDR). These transfected cultures were incubated with 1.alpha.,24-(OH).sub.2D.sub.4 or 1.alpha.,25-(OH).sub.2D.sub.3, and the production of growth hormone was measured. Table. . .

DETD

TABLE 2

Induction of Growth Hormone by **Vitamin D** Compounds

Compound Used	Concentration (M)	Growth Hormone Induction (ng/ml)
1,25-(OH).sub.2D.sub.3	1 .times. 10.sup.-10	39
1,25-(OH).sub.2D.sub.3	5 .times. 10.sup.-10	248
1,24-(OH).sub.2D.sub.4	5 .times. 10.sup.-10	165

1,24-(OH).sub.2D.sub.4. . .

DETD These data show that the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to stimulate **vitamin D**-dependent growth hormone is nearly equivalent to that of 1.alpha.,25-(OH).sub.2D.sub.3. Such results are truly surprising and would not have been expected. . .

DETD 1.alpha.,24(S)-Dihydroxyvitamin D.sub.2 and 1.alpha.,24(R)-Dihydroxy-**vitamin D.sub.2** [1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2]

DETD . . . was conducted to compare the biological activity in vitro of chemically synthesized 1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2, with 1.alpha.,25-(OH).sub.2D.sub.3 and 25-OH-D.sub.3. The **vitamin D**-dependent transcriptional activation model system was used in which plasmids pSG5-hVDR1/3 and p(CT4).sup.4TKGH were co-transfected into Green monkey

kidney, COS-1 cells.

DETD Transfected cells were incubated with **vitamin D** metabolites and growth hormone production was measured. As shown in Table 3, both 1.alpha.,24(S)-(OH).sub.2D.sub.2 and its epimer, 1.alpha.,24(R)-(OH).sub.2D.sub.2, had significantly. . .

DETD

TABLE 3

Vitamin D-Inducible Growth Hormone Production In Transfected COS-1 Cells

Vitamin DCInducible Growth Hormone Production Net Molar Con- Inducer.	Total GH Production*	vitamin DCinducible GH-production

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. Medium containing test analogue or vehicle is replaced every three. . .

DETD 1.alpha.,24-Dihydroxy **Vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue, 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. After 6-7 days, the medium is removed and stored at. . .

DETD The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,24-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD 1.alpha.,25-Dihydroxy **Vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,25-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD Patients with a known **vitamin D** receptor positive tumor (e.g., adenocarcinoma of the prostate, breast, **lung**, colon or pancreas, or transitional cell carcinoma of the bladder, or melanoma) participate in an open-label study of a hypocalcemic **vitamin D** compound in accordance with the present invention. Patients are placed on a reduced calcium diet prior to treatment, to help minimize intestinal absorption and allow ever higher doses of the hypocalcemic **vitamin D**. This reduced calcium diet may be continued for the duration of treatment, and for one week after the last dose. . . of the 1.alpha.,24(S)-dihydroxyvitamin D.sub.2. The diet ideally restricts daily calcium intake to 400-500 mg. Patients also discontinue use of any **vitamin D** supplements or **vitamin D** replacement therapies. Each

patient is also asked to drink 4-6 cups of fluid more than usual intake to assure adequate. . .

DETD Treatment of Prostate **Cancer** with 1.alpha.,24-Dihydroxy **Vitamin D.sub.2** [1.alpha.,24-(OH).sub.2D.sub.2]

DETD Patients with advanced androgen-independent prostate **cancer** participate in an open-labeled study of 1.alpha.,24-(OH).sub.2D.sub.2. Qualified patients are at least 40 years old, exhibit histologic evidence of adenocarcinoma. . . patients begin a course of therapy with oral 1.alpha.,24-(OH).sub.2D.sub.2 lasting 26 weeks, while discontinuing any previous use of calcium supplements, **vitamin D** supplements, and **vitamin D** hormone replacement therapies. During treatment, the patients are monitored at regular intervals for: (1) hypercalcemia, hyperphosphatemia, hypercalciuria, hyperphosphaturia and other. . .

DETD Treatment of Prostate **Cancer** with 1.alpha.-Hydroxy **Vitamin D.sub.2** [1.alpha.-OH-D.sub.2]

DETD The study of Example 14 is repeated for the active **vitamin D** compound, 1.alpha.-OH-D.sub.2. The results of the phase one study indicate that patients treated with the MTD of 1.alpha.-OH-D.sub.2 for at. . .

DETD Treatment of Liver **Cancer**

CLM What is claimed is:

. . . antiproliferative amount of a hypocalcemic hydroxyvitamin D compound having a hydrocarbon moiety at the C.sub.24 position, the cells expressing a **vitamin D** receptor.

2. A method in accordance with claim 1, wherein the cells are cancers of the breast, colon, **lung**, neck and head, pancreas, endometrium, bladder, cervix, testes, ovaries, and liver, squamous cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary. . .

3. A method in accordance with claim 1, wherein the hypocalcemic **vitamin D** is a compound represented by formula (I) ##STR4## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon bond, thus. . .

4. A method in accordance with claim 1 wherein the hypocalcemic **vitamin D** compound is a compound of formula II ##STR5## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon bond, thus. . .

5. A method in accordance with claim 1, wherein the hypocalcemic **vitamin D** compound is a compound of formula III: ##STR6## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon bond, thus. . .

7. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is administered in a daily regimen or an episodic regimen.

9. A method in accordance with claim 7, wherein the hypocalcemic **vitamin D** compound is administered daily at a dose of about 10 to 100 .mu.g/day.

10. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is administered orally, is administered intravenously, is directly injected to a **cancer** site or is regionally delivered to a **cancer** site.

11. A method in accordance with claim 10, wherein the hypocalcemic **vitamin D** compound is administered orally.

12. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is co-administered with a cytotoxic

agent.

25. A method of treating a human to alleviate the pathological effects of breast **cancer**, colon **cancer**, testicular **cancer**, pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . .

26. A method of claim 25, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula (III) ##STR7## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon. . .

. . . a disease in need of treatment by a cytotoxic agent, comprising administering to the patient a therapeutic amount of hypocalcemic **vitamin D** compound and the cytotoxic agent.

30. A method in accordance with claim 29, wherein the hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of the cytotoxic agent.

31. A method in accordance with claim 29, wherein the hypocalcemic **vitamin D** compound is administered 2 to 4 days prior to administration of the cytotoxic agent.

32. A method of claim 29, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula (III) ##STR8## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon. . .

33. The method of claim 32, wherein said therapeutic amount of the **vitamin D** compound is 0.01 .mu.g/kg/day to 2.0 .mu.g/kg/day.

. . . of inducing differentiation in malignant or neoplastic cells, comprising treating to the cells with a prodifferentiative amount of a hypocalcemic **vitamin D** compound.

37. A method of treating in a subject tumor or neoplasm that expresses a **vitamin D** receptor, comprising administering to the subject an effective amount of hypocalcemic **vitamin D** compound sufficient to raise a blood level of **vitamin D** to a sufficiently supraphysiological level for a sufficient period of time to inhibit growth of the tumor or neoplasm without. . .

38. A method in accordance with claim 7, wherein the hypocalcemic **vitamin D** compound is administered episodically at a dose of about 10 .mu.g to 200 .mu.g/dose.

39. A method in accordance with claim 8, wherein the hypocalcemic **vitamin D** compound is administered at a dose of about 10 .mu.g to 200 .mu.g/dose.

44. A method in accordance with claim 29, wherein the therapeutic amount of the hypocalcemic **vitamin D** compound is 10 .mu.g to 200 .mu.g/dose.

L4 ANSWER 11 OF 12 USPAT2
AN 2002:32553 USPAT2
TI Method of inhibiting angiogenesis using active **vitamin D** analogues
IN Bishop, Charles W., Madison, WI, United States
Mazess, Richard B., Madison, WI, United States

PA Bone Care International, Inc., Middleton, WI, United States (U.S. corporation)
PI US 6573256 B2 20030603
AI US 2001-891805 20010626 (9)
RLI Continuation-in-part of Ser. No. US 1998-596149, filed on 23 Feb 1998 Division of Ser. No. US 1996-781910, filed on 30 Dec 1996, now patented, Pat. No. US 5763429
DT Utility
FS GRANTED
EXNAM Primary Examiner: Criares, Theodore J.
LREP Michael Best & Friedrich LLP, Welch, Teresa J., Peterson, Jeffrey D.
CLMN Number of Claims: 34
ECL Exemplary Claim: 1
DRWN 0 Drawing Figure(s); 0 Drawing Page(s)
LN.CNT 1221
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Method of inhibiting angiogenesis using active **vitamin D** analogues
AB Methods utilizing active **vitamin D** analogs for the inhibition of angiogenesis associated with malignant and neoplasmic cells. Methods comprise the application of an effective amount of a hypocalcemic **vitamin D** compound to inhibit the angiogenesis of malignant cells, inducing the apoptosis of malignant cells, and regressing the growth of tumorous. . .
SUMM . . . of inhibiting angiogenesis associated with the hyperproliferation of malignant cells, and in particular, to the use of active forms of **vitamin D** to inhibit angiogenesis of malignant cells.
SUMM Extensive research during the past two decades has established important biologic roles for **vitamin D** apart from its classic role in bone and mineral metabolism. Specific nuclear receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3, the hormonally active form of **vitamin D**, are present in cells from diverse organs not involved in calcium homeostasis. For example, specific, biologically active **vitamin D** receptors have been demonstrated in the human prostatic carcinoma cell line, LNCaP, (Miller et al., 52 **Cancer** Res. (1992) 515-520); **Vitamin D** receptors have also been described for many other neoplastic cells, e.g., carcinomas of the breast and the colon.
SUMM It has been reported that certain **vitamin D** compounds and analogues are potent inhibitors of malignant cell proliferation and are inducers/stimulators of cell differentiation. For example, U.S. Pat.. . . nonmalignant macrophages (monocytes), and are useful in the treatment of leukemia. Antiproliferative and differentiating actions of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues have been reported with respect to **cancer** cell lines. More recently, an association between **vitamin D** receptor gene polymorphism and **cancer** risk has been reported, suggesting that **vitamin D** receptors may have a role in the development, and possible treatment, of **cancer**.
SUMM These previous studies have focused exclusively on **vitamin D**.sub.3 compounds. Even though these compounds may indeed be highly effective in promoting differentiation in malignant cells in culture, their practical. . . blood calcium levels by virtue of their inherent calcemic activity. That is, the clinical use of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues as anticancer agents is precluded, or severely limited, by the risk of hypercalcemia.
SUMM . . . the regression was due to the induction of apoptosis within the cell population. As mentioned earlier however, use of such **vitamin D**.sub.3 analogs as anticancer agents is

limited due to the inherent calcemic activity of the compounds. Therefore a need exists for. . .

SUMM The present invention provides a method of inhibiting angiogenesis associated with malignant cells. The method includes use of hypocalcemic active **vitamin D** compounds to inhibit angiogenesis. The present invention also provides a method of inducing the apoptosis of **cancer** cells by the use of active **vitamin D** compounds, and includes a method for treating **cancer** by regressing tumor cells by the use of active **vitamin D** compounds.

SUMM . . . a method of inhibiting angiogenesis associated with malignant cells, comprising treating the cells with an effective amount of a hypocalcemic **vitamin D** compound. The hypocalcemic **vitamin D** compound of the present invention include hypocalcemic **vitamin D** compounds having a hydrocarbon moiety substituted at the C-24 position on the sidechain of the molecule and having a hydroxyl. . .

SUMM The hypocalcemic **vitamin D** compound is an active **vitamin D** and is suitably represented by the formula (I) described hereafter. Suitable compounds of formula (I), are 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4,. . .

SUMM In another aspect of the invention, the apoptosis of **cancer** cells is accomplished by a method comprising, administering to patients an effective amount of a hypocalcemic **vitamin D** compound to induce the apoptosis of **cancer** cells.

SUMM In yet another aspect of the invention, a method for treating **cancer** by regressing tumor cells is disclosed, comprising administering to patients an effective amount of a hypocalcemic **vitamin D** compound to induce the regression of **cancer** cells.

SUMM In accordance with the present invention, when effective amounts of the hypocalcemic **vitamin D** compounds are administered to patients with **cancer** or neoplasms, the proliferative activity of the abnormal neoplastic cells is inhibited or maintained, and cell differentiation is induced, promoted or enhanced, with significantly less hypercalcemia and hypercalciuria than is observed after the same amount of an activated **vitamin D**.sub.3 (e.g., 1.alpha.--OH D.sub.3, 1.alpha.,25--(OH).sub.2 D.sub.3) is administered in previously known formulations. Thus, the compound in accordance with the present invention has an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues. Furthermore, the compounds of the present invention can be administered in doses significantly higher than that of active **vitamin D**.sub.3 analogs due to their lower calcemic effect.

SUMM Accordingly, another aspect of the invention is a method of treating human **cancer** comprising administering to a subject who has **cancer** an effective amount of active **vitamin D** compound which has, attained through metabolism in vivo, a **vitamin D** receptor (VDR) binding affinity substantially equivalent to the binding affinity of 1.alpha.,25-dihydroxyvitamin D.sub.3 and a hypercalcemia risk substantially lower that that of 1.alpha.,25-dihydroxyvitamin D.sub.3, to decrease or stabilize the cellular abnormal proliferative activity of the **cancer**.

SUMM For treatment for malignant conditions in accordance with the present invention, the active **vitamin D** is suitably administered alone as an active ingredient in a pharmaceutical composition, or is co-administered with an anticancer agent.

SUMM Further, included within the scope of the present invention is the co-administration of a hypocalcemic **vitamin D** compound with a cytotoxic or anticancer agent. Such agents suitably

include antimetabolites (e.g., 5-fluoro-uracil, methotrexate, fludarabine), antimicrotubule agents (e.g., vincristine,. . .

SUMM It is anticipated that the active **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

SUMM Also included within the scope of the present invention is the co-administration of effective dosages of a hypocalcemic **vitamin D** compound in conjunction with administration of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. For example, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

SUMM In another aspect, the invention is a pharmaceutical composition which includes an anticancer agent which is an active **vitamin D** compound; an agent selected from the group consisting of (i) an anticancer agent, (ii) a bone agent, and combinations thereof;. . .

DETD . . . present invention provides a novel inhibition of angiogenesis of a patient suffering from a hyperproliferative disease with an active hypocalcemic **vitamin D** compound. The active **vitamin D** analogue is suitably a hydroxyvitamin D compound e.g. a 1.alpha.-hydroxy **vitamin D** or a 24-hydroxy **vitamin D**, and is suitably represented by formula (I) as described hereinbelow. The active **vitamin D** analogue is provided to the patient without causing dose-limiting hypercalcemia and hypercalciuria, i.e., unphysiologically high and deleterious blood calcium levels. . . in fact reduces the hypercalcemia caused by the malignancy. These attributes are achieved through specific chemical properties of the hypocalcemic **vitamin D** compounds as described.

DETD In accordance with the present invention, when effective amounts of the analogues of the hypocalcemic **vitamin D** compound are administered to patients with malignant diseases, the angiogenesis of cancerous cells is inhibited, tumorous cells are regressed, cancerous. . . is induced, promoted or enhanced, with significantly less hypercalcemia and hypercalciuria than is observed after the same amount of activated **vitamin D**.sub.3 is administered in previously known formulations. Thus, the hypocalcemic **vitamin D** compounds of the present invention have an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues.

DETD It is known that **vitamin D**.sub.3 must be hydroxylated in the C-1 and C-25 positions before it is activated, i.e., before it will produce a biological response. A similar metabolism appears to be required to activate other forms of **vitamin D**, e.g., **vitamin D.sub.2** and **vitamin D.sub.4**. Therefore, as used herein, the term "activated **vitamin D**" or "active **vitamin D**" is intended to refer to a **vitamin D** compound or analogue that has been hydroxylated in at least the C-1, C-24 or C-25 position of the molecule and either the compound itself or its metabolites in the case of a prodrug, such as 1.alpha.-hydroxyvitamin D.sub.2, binds the **vitamin D** receptor (VDR). For example, "prodrugs" include **vitamin D** compounds which are hydroxylated in the C-1 position. Such compounds undergo further hydroxylation in vivo and their metabolites bind the. . .

DETD The term "hypocalcemic **vitamin D** compound" is in reference to active **vitamin D** analogs which demonstrate hypocalcemic activity, i.e., substantially less calcemic activity relative to the calcemic activity of 1.alpha.,25-dihydroxy **vitamin D**.sub.3. Such compounds include

24-hydroxyvitamin D compounds, 25-hydroxyvitamin D compounds and 1.alpha.-hydroxyvitamin D compounds.

DETD The compound in accordance with the present invention is an active hypocalcemic **vitamin D** compound. The active **vitamin D** provided is such that the compound has a hydrocarbon moiety at the C-24 position, e.g. a lower alkyl, alkenyl or acyl group as the C-24 position. Further, the active **vitamin D** in accordance with the present invention may have an unsaturated sidechain, e.g., there is suitably a double bond between C-22. . .

DETD The hypocalcemic **vitamin D** compounds of formula (I) of the present invention are those that have an effective inhibition effect on the angiogenesis of. . . to malignant or other hyperproliferative cells without significantly altering calcium metabolism. This selectivity and specificity of action makes the hypocalcemic **vitamin D** compounds useful and preferred agents for safely inhibiting angiogenesis of hyperproliferative cells. The compounds of the present invention, thus, overcome the shortcomings of the known active **vitamin D**.sub.3 compounds described above, and can be considered preferred agents for the control angiogenesis of malignant diseases such breast, colon, testicular and prostate **cancer**, as well as other neoplasms such as pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . medullary thyroid carcinoma, multiple myeloma, melanoma, retinoblastoma, and sarcomas of the soft tissue and bone, i.e., neoplasms that express a **vitamin D** receptor.

DETD Suitable active **vitamin D** compounds of formula (I) include: 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin D.sub.2, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.-hydroxyvitamin D.sub.2, and 1.alpha.-hydroxyvitamin D.sub.4. Among those. . .

DETD . . . of malignant cells as well as other hyperproliferative cells such as psoriatic cells with an effective amount of a hypocalcemic **vitamin D** compound. The effective dosage amount on a daily basis per kilogram of body weight of the patient ranges from about. . . dose is given. The compounds in accordance with the present invention are administered in an amount that raises a serum **vitamin D** level to a supraphysiological level for a sufficient time to inhibit angiogenesis or induce the hypercalcemic properties of the compounds. . .

DETD The compounds of formula (I) are valuable for the inhibition of angiogenesis of **cancer** and neoplasms in a patient suffering therefrom. In particular, the invention is a method for treating a patient suffering from the hyperproliferative cellular effects of **cancer** and othe neoplasms by administering to the patient a therapeutically effective amount of a compound of formula (I), which is. . . suitably 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin D.sub.2, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.-hydroxyvitamin D.sub.2, and 1.alpha.-hydroxyvitamin D.sub.4, sufficient to inhibit antiogenesis of the **cancer** neoplasms. Among those compounds of formula (I) that have a chiral center in the sidechain, such as at C-24, it. . .

DETD . . . the compounds of formula (I) have been studied and compared to that of 1.alpha.,25-dihydroxyvitamin D.sub.3, the active hormonal form of **vitamin D** and the standard against which all **vitamin D** compounds and analogues are measured. For example, it has been found that the **vitamin D** receptor (VDR) binding affinities of the compounds of formula (I), or

their active metabolites, are substantially equivalent to (i.e., equal. . .

DETD At the same time, it has been found that compounds of formula (I) are significantly less toxic than their corresponding **vitamin D**.sub.3 analogues. For example, in parent co-pending application, Ser. No. 08/265,438, the disclosure of which is incorporated herein by reference, the. . .

DETD The hypocalcemic **vitamin D** compounds are useful as active compounds or ingredients in pharmaceutical compositions having reduced side effects and low toxicity as compared with the known analogues of active forms of **vitamin D**.sub.3.

DETD . . . conventional methods of pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans. For example, the hypocalcemic **vitamin D** compounds of the present invention can be employed in admixtures with conventional excipients, e.g., pharmaceutically acceptable carrier substances suitable for. . .

DETD . . . wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or one or more other active compounds, for example, **vitamin D**.sub.3 and its 1.alpha.-hydroxylated metabolites, conjugated estrogens or their equivalents, anti-estrogens, calcitonin, biphosphonates, calcium supplements, cobalamin, pertussis toxin and boron.

DETD . . . is about 0.01 .mu.g to about 50 .mu.g per gram of composition. For treatment of cancers, the dosage of hypocalcemic **vitamin D** compound in a locally applied composition generally is about 0.01 .mu.g to 100 .mu.g per gram composition.

DETD . . . administration of the pharmaceutical compositions of the present invention is preferred. The dosage of the compounds for the treatment of **cancer** or neoplasms according to this invention generally is about 0.01 to about 2.0 .mu.g/kg/day, preferably about 0.01 to about 1.0 .mu.g/kg/day. As noted above, dosing of the hypercalcemia **vitamin D** compounds in accordance with the present invention can be done on an episodic basis in which higher doses can be. . .

DETD Further, included within the scope of the present invention is the co-administration of hypocalcemic **vitamin D** compound with an anticancer agent, e.g., a cytotoxic agent, Such agents suitably include antimetabolites (e.g., 5-fluoro-uracil, methotrexate, fludarabine), antimicrotubule agents. . . daunomycin), topoisomerase inhibitors (e.g., etoposide, camptothecins) or any other cytotoxic agents. (estramustine phosphate, prednimustine). It is anticipated that the hypocalcemic **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

DETD . . . other at a later time, typically within a week. An example of a suitable co-administration regimen is where a hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of a cytotoxic agent.

DETD Also included within the scope of the present invention is the co-administration of effective dosages of the hypocalcemic **vitamin D** compounds in conjunction with administration of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. For example, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

DETD The affinity of 1.alpha.,24-(OH).sub.2D.sub.2 for the mammalian **vitamin D** receptor (VDR) was assessed using a commercially available kit of bovine thymus VDR and standard 1 ,25-(OH).sub.2D.sub.3 solutions from lncstar. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.**

4 [1.alpha.,24-(OH).sub.2D.sub.4]

DETD The VDR affinity binding of 1.alpha.,24-(OH).sub.2D.sub.4 was investigated. The 1.alpha.,24-(OH).sub.2D.sub.4 was incubated with **vitamin D** receptor and radiolabeled tracer 1.alpha.,25-(OH).sub.2D.sub.3. After incubation, the amount of radioactivity bound to the receptor was determined and compared with. . .

DETD These results show that 1.alpha.,24-(OH).sub.2D.sub.4 binds slightly less tightly to the **vitamin D** receptor than does 1.alpha.,25-(OH).sub.2D.sub.3. Such data mean that 1.alpha.,24-(OH).sub.2D.sub.4 has high affinity for the VDR and significant biological activity, similar. . .

DETD . . . results are surprising and unexpected in view of the prior art. They are contrary to the normative wisdom in the **vitamin D** art regarding the very low degree of biological activity of **vitamin D.sub.4** compounds.

DETD VDR binding of **vitamin D** compounds by prostate cells is demonstrated using the techniques of Skowronski et al., 136 Endocrinology (1995) 20-26, which is incorporated. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,24-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. .

DETD The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,25-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD Using the plasmids p(CT4).sup.4TKGH, a **vitamin D** receptor (VDR)-expressing plasmid, and pSG5-hVDR1/3, a plasmid containing a Growth Hormone (GH) gene, under the control of a **vitamin D**-responsive element (VDRE), experiments were conducted to explore the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to induce **vitamin D**-dependent growth hormone acting as a reporter gene compared to that of 1.alpha.,25-(OH).sub.2D.sub.3. Cells in culture were transfected with these two plasmids. One plasmid contained the gene for Growth Hormone (GH) under the control of the **vitamin D** responsive element (VDRE) and the other plasmid contained the structural gene for the **vitamin D** receptor (VDR). These transfected cultures were incubated with 1.alpha.,24-(OH).sub.2D.sub.4 or 1.alpha.,25-(OH).sub.2D.sub.3, and the production of growth hormone was measured. Table. . .

DETD

TABLE 2

Induction of Growth Hormone by **Vitamin D** Compounds

Compound Used	Concentration (M)	Growth Hormone Induction (ng/ml)
1,25-(OH).sub.2D.sub.3	1 .times. 10.sup.-10	39
1,25-(OH).sub.2D.sub.3	5 .times. 10.sup.-10	248
1,24-(OH).sub.2D.sub.4	5 .times. 10.sup.-10	165

1,24-(OH).sub.2D.sub.4. . .

DETD These data show that the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to stimulate **vitamin D**-dependent growth hormone is nearly equivalent to that of 1.alpha.,25-(OH).sub.2D.sub.3. Such results are truly surprising and would not have been expected. . .

DETD 1.alpha.,24(S)-dihydroxyvitamin D.sub.2 and 1.alpha.,24(R)-dihydroxy-**vitamin D.sub.2**
[1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2]

DETD . . . was conducted to compare the biological activity in vitro of chemically synthesized 1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2, with 1.alpha.,25-(OH).sub.2D.sub.3 and 25-OH-D.sub.3. The **vitamin D**-dependent transcriptional activation model system was used in which plasmids pSG5-hVDR1/3and p(CT4).sup.4TKGH were co-transfected into Green monkey kidney, COS-1 cells.

DETD Transfected cells were incubated with **vitamin D** metabolites and growth hormone production was measured. As shown in Table 3, both 1.alpha.,24(S)-(OH).sub.2D.sub.2 and its epimer, 1.alpha.,24(R)-(OH).sub.2D.sub.2, had significantly. . .

DETD
TABLE 3

Vitamin D-Inducible Growth Hormone Production
In Transfected COS-1 Cells
Vitamin DCInducible Growth
Hormone Production
Net vitamin
Total GH DCinducible
Molar Production* GH-production
Inducer Concentration. . .

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. Medium containing test analogue or vehicle is replaced every three. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue, 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. After 6-7 days, the medium is removed and stored at. . .

DETD The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,24-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,25-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD A **vitamin D** compound of formula (I) inhibition of VEGF-induced endothelial cell proliferation is demonstrated using the techniques of Mantell et al., Circulation. . .

CLM What is claimed is:
. . . inhibiting angiogenesis associated with malignant or neoplastic cells, comprising treating the cells with an effective amount of a hypocalcemic hydroxy **vitamin D** compound having a

hydrocarbon moiety at the C.sub.24 position, the cells being cancers of the breast, colon, prostate, testes, **lung**, neck and head, pancreas, endometrium, bladder, cervix, ovaries, squamous cell carcinomas, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma, melanoma,. . .

. . . malignancy or neoplasm in a subject in need thereof, comprising administering to the subject an effective amount of a hypocalcemic **vitamin D** compound represented by formula I: ##STR4## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon--carbon bond, thus forming a. . .

3. A method in accordance with claim 2, wherein the hypocalcemic **vitamin D** compound is represented by formula II: ##STR5## wherein A.sup.1 and A.sup.2each are hydrogen or a carbon--carbon bond, thus forming a. . .

4. The method of claim 2, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound is represented by formula III: ##STR6## wherein A.sup.1 and A.sup.2 each are hydrogen or a. . .

6. A method in accordance with claim 2, wherein a dosing regimen for the hypocalcemic **vitamin D** compound is a daily regimen or an episodic regimen.

8. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is administered daily at a dose of about 10 to 100 .mu.g/day.

9. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is administered orally, is administered intravenously, is injected directly into a **cancer** site, or is regionally delivered to a **cancer** site.

10. A method in accordance with claim 9, wherein the hypocalcemic **vitamin D** compound is administered orally.

11. A method in accordance with claim 2, wherein the hypocalcemic **vitamin D** compound is co-administered with a cytotoxic agent.

22. A method of treating a human to inhibit angiogenesis associated with breast **cancer**, colon **cancer**, prostate **cancer**, testicular **cancer**, pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid and lymphocyltic leukemia, lymphoma,. . . retinoblastoma or sarcomas of the soft tissue and bone, comprising administering to the human an effective amount of a hypocalcemic **vitamin D** compound.

23. A method of claim 22, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula III: ##STR7## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon--carbon. . .

. . . A method of treating a human to inhibit angiogenesis associated with malignant cells, comprising administering to the patient a hypocalcemic **vitamin D** compound and a cytotoxic agent.

27. A method in accordance with claim 26, wherein the hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of the cytotoxic agent.

28. A method in accordance with claim 26, wherein the hypocalcemic

vitamin D compound is administered 2 to 4 days prior to administration of the cytotoxic agent.

29. A method of claim 26, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula III: ##STR8## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon--carbon. . .

. . . method of inducing apoptosis of malignant or neoplastic cells, comprising treating the cells with an effective amount of a hypocalcemic **vitamin D** compound, the cells being cancers of the breast, colon, prostate, testes, **lung**, neck and head, pancreas, endometrium, bladder, cervix, ovaries, squamous cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma, melanoma,. . .

. . . A method of inducing the regression of tumor cells comprising treating the cells with an effective amount of a hypocalcemic **vitamin D** compound, which inhibits angeogenesis associated with malignancy the cells being cancers of the breast, colon, prostate, testes, **lung**, neck and head, pancreas, endometrium, bladder, cervix, ovaries, squamous cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma, melanoma,. . .

L4 ANSWER 12 OF 12 USPAT2
AN 2002:17285 USPAT2
TI Method of treating malignancy associated hypercalcemia using active **vitamin D** analogues
IN Bishop, Charles W., Madison, WI, United States
Mazess, Richard B., Madison, WI, United States
PA Bone Care International, Inc., Middleton, WI, United States (U.S. corporation)
PI US 6566353 B2 20030520
AI US 2001-891763 20010626 (9)
RLI Continuation-in-part of Ser. No. US 1998-596149, filed on 23 Feb 1998 Division of Ser. No. US 1996-781910, filed on 30 Dec 1996, now patented, Pat. No. US 5763429
DT Utility
FS GRANTED
EXNAM Primary Examiner: Criares, Theodore J.
LREP Michael Best & Friedrich LLP, Welch, Teresa J., Peterson, Jeffrey D.
CLMN Number of Claims: 33
ECL Exemplary Claim: 1
DRWN 0 Drawing Figure(s); 0 Drawing Page(s)
LN.CNT 1147
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Method of treating malignancy associated hypercalcemia using active **vitamin D** analogues
AB Methods utilizing active **vitamin D** analogs for the treatment of malignancy-associated hypercalcemia. Methods comprise the application of an effective amount of a hypocalcemic **vitamin D** compound to alleviate hypercalcemia, lower serum parathyroid hormone related protein (PTHrP) levels.
SUMM . . . relates generally to a method of treating malignancy-associated hypercalcemia (MAH), and in particular, to the use of active forms of **vitamin D** to reduce hypercalcemia associated with inhibit the hyperproliferative diseases.
SUMM Extensive research during the past two decades has established important biologic roles for **vitamin D** apart from its classic role in bone and mineral metabolism. Specific nuclear receptors for 1.alpha.,25-dihydroxyvitamin D.sub.3, the hormonally active form of **vitamin D**, are present in cells from diverse organs not involved in calcium homeostasis. For example, specific, biologically

active **vitamin D** receptors have been demonstrated in the human prostatic carcinoma cell line, LNCaP, (Miller et al., 52 **Cancer** Res. (1992) 515-520); **Vitamin D** receptors have also been described for many other neoplastic cells, e.g., carcinomas of the breast and carcinomas of the colon.

SUMM It has been reported that certain **vitamin D** compounds and analogues are potent inhibitors of malignant cell proliferation and are inducers/stimulators of cell differentiation. For example, U.S. Pat.. . . nonmalignant macrophages (monocytes), and are useful in the treatment of leukemia. Antiproliferative and differentiating actions of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues have been reported with respect to **cancer** cell lines. More recently, an association between **vitamin D** receptor gene polymorphism and **cancer** risk has been reported, suggesting that **vitamin D** receptors may have a role in the development, and possible treatment, of **cancer**.

SUMM These previous studies have focused exclusively on **vitamin D**.sub.3 compounds. Even though these compounds may indeed be highly effective in promoting differentiation in malignant cells in culture, their practical. . . blood calcium levels by virtue of their inherent calcemic activity. That is, the clinical use of 1.alpha.,25-dihydroxyvitamin D.sub.3 and other **vitamin D**.sub.3 analogues as anticancer agents is precluded, or severely limited, by the risk of hypercalcemia.

SUMM . . . themselves increase serum calcium levels. Therefore a need exists for compounds with greater specific activity and selectivity of action, i.e., **vitamin D** compounds with antiproliferative and differentiating effects but which have less calcemic activity.

SUMM . . . hypercalcemia (MAH) such as that associated with hyperproliferative cell growth and/or abnormal cell differentiation. The method includes use of active **vitamin D** compounds to treat hypercalcemia and reduce serum parathyroid hormone related protein (PTHrP) levels.

SUMM A hydroxyvitamin D compound in accordance with the present invention is an active **vitamin D** and is suitably represented by the formula (I) described hereafter. Suitable compounds of formula (I) are 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4,. . .

SUMM . . . patients suffering from hypercalcemia is accomplished by a method comprising, administering to these patients an effective amount of a hypocalcemic **vitamin D** compound, to lower the serum parathyroid hormone related protein (PTHrP) level.

SUMM The hypocalcemic **vitamin D** compounds are also valuable for the treatment of breast, prostate and colon **cancer**, as well as other neoplasms such as pancreatic **cancer**, endometrial **cancer**, testicular **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . tumors, medullary thyroid carcinoma, multiple myeloma, retinoblastoma, and sarcomas of the soft tissue and bone, i.e. neoplasms that express a **vitamin D** receptor.

SUMM In accordance with the present invention, when effective amounts of the hypocalcemic **vitamin D** compounds are administered to patients with MAH, significantly redeuced hypercalcemia is observed than is observed after the same amount of an activated **vitamin D**.sub.3 (e.g., 1.alpha.-OH D.sub.3, 1.alpha.,25-(OH).sub.2 D.sub.3) is administered in previously known formulations. Thus, the compound in accordance with the present invention has an improved therapeutic index relative to active forms of **vitamin**

D.sub.3 analogues.

SUMM . . . method of treating malignancy associated hyercalcemia comprising administering to a subject who is suffering therefrom an effective amount of active **vitamin D** compound which has, or attains through metabolism in vivo, a **vitamin D** receptor (VDR) binding affinity substantially equivalent to the binding affinity of 1.alpha.,25-dihydroxyvitamin D.sub.3 and has a hypercalcemia risk substantially lower. . .

SUMM For treatment for malignancy-associated hypercalcemia and the underlying malignant condition in accordance with the present invention, the active **vitamin D** is suitably administered alone as an active ingredient in a pharmaceutical composition, or is co-administered with an anticancer agent.

SUMM Further, included within the scope of the present invention is the co-administration of a hypocalcemic **vitamin D** compound with a cytotoxic or anticancer agent. Such agents suitably include antimetabolites (e.g., 5-fluoro-uracil, methotrexate, fludarabine), antimicrotubule agents (e.g., vincristine,. . .

SUMM It is anticipated that the active **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

SUMM Also included within the scope of the present invention is the co-administration of effective dosages of a hypocalcemic **vitamin D** compound in conjunction with administration of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. For example, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

SUMM In another aspect, the invention is a pharmaceutical composition which includes an anticancer agent which is an active hypocalcemic **vitamin D** compound; an agent selected from the group consisting of (i) an anticancer agent, (ii) a bone agent, and combinations thereof;. . .

SUMM . . . cells. The present invention provides a novel treatment of a patient suffering from a hyperproliferative disease with an active hypocalcemic **vitamin D** compound. Preferably, the active **vitamin D** analogue is a hydroxyvitamin D compound and is suitably represented by formula (I) as described hereinbelow. The active **vitamin D** analogue is provided to the patient without itself causing dose-limiting hypercalcemia and hypercalciuria, and in fact, reduces the hypercalcemia caused by the malignancy. These attributes are achieved through specific chemical properties of the hypocalcemic **vitamin D** compounds as described.

SUMM In accordance with the present invention, when effective amounts of the hypocalcemic active **vitamin D** compounds are administered to patients with malignant diseases, the hypercalcemia is reduced, the PTHrP serum level is reduced, and the. . . of the abnormal cells is inhibited, redeuced, or stabilized, and cell differentiation is induced, promoted or enhanced. Thus, the hypocalcemic **vitamin D** compounds of the present invention have an improved therapeutic index relative to active forms of **vitamin D**.sub.3 analogues.

SUMM It is known that **vitamin D**.sub.3 must be hydroxylated in the C-1 and C-25 positions before it is activated, i.e., before it will produce a biological response. A similar metabolism appears to be required to activate other forms of **vitamin D**, e.g., **vitamin D.sub.2** and **vitamin D.sub.4**. Therefore, as used herein, the term "activated **vitamin D**" or "active **vitamin D**" is intended to refer to a

vitamin D compound or analogue that has been hydroxylated in at least the C-1, C-24 or C-25 position of the molecule and either the compound itself or its metabolites in the case of a prodrug, such as 1.alpha.-hydroxyvitamin D.sub.2, binds the **vitamin D** receptor (VDR). For example, "prodrugs" are **vitamin D** compounds which are hydroxylated in the C-1. Such compounds undergo further hydroxylation in vivo and their metabolites bind the VDR.

SUMM The term "hypocalcemic **vitamin D** compound" is in reference to active **vitamin D** analogs which demonstrate hypocalcemic activity, i.e. have low calcemic activity relative to that of 1.alpha.,25-dihydroxyvitamin D.sub.3, including 24-hydroxyvitamin D compounds,. . .

SUMM The compound in accordance with the present invention is an active hypocalcemic **vitamin D** compound. The active **vitamin D** provided is such that the compound has a hydrocarbon moiety at the C-24 position, e.g. a lower alkyl, alkenyl or acyl group as the C-24 position. Further, the active **vitamin D** in accordance with the present invention may have an unsaturated sidechain, e.g., there is suitably a double bond between C-22. . .

SUMM . . . other hyperproliferative cells and can reduce hypercalcemia associated with the maligancy. This selectivity and specificity of action makes the hypocalcemic **vitamin D** compounds useful and preferred antihypercalcemic agents as well as safely inhibiting hyperproliferation and promoting malignant or hyperplastic cell differentiation. The compounds of the present invention, thus, overcome the shortcomings of the known active **vitamin D**.sub.3 compounds described above, and can be considered preferred agents for the control and treatment of malignant diseases such breast, prostate, testicular and colon **cancer**, as well as other neoplasms such as pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . tumors, medullary thyroid carcinoma, multiple myeloma, melanoma retinoblastoma, and sarcomas of the soft tissue and bone, i.e. neoplasms that express **vitamin D** receptors.

SUMM Suitable hypocalcemic **vitamin D** compounds in accordance with the present invention include: 1.alpha.,24-dihydroxyvitamin D.sub.2, 1.alpha.,24-dihydroxyvitamin D.sub.4, 1.alpha.,25-dihydroxyvitamin D.sub.2, 1.alpha.,25-dihydroxyvitamin D.sub.4, 1.alpha.-hydroxyvitamin D.sub.2, and 1.alpha.-hydroxyvitamin. . .

SUMM . . . the present invention provides a method of treating hypercalcemia associated with malignant cells with an effective amount of a hypocalcemic **vitamin D** compound. The effective dosage amount on a daily basis per kilogram of body weight of the patient ranges from about. . .

SUMM . . . the compounds of formula (I) have been studied and compared to that of 1.alpha.,25-dihydroxyvitamin D.sub.3, the active hormonal form of **vitamin D** and the standard against which all **vitamin D** compounds and analogues are measured. For example, it has been found that the **vitamin D** receptor (VDR) binding affinities of the compounds of formula (I), or their active metabolites, are substantially equivalent to (i.e., equal. . .

SUMM At the same time, it has been found that compounds of formula (I) are significantly less toxic than their corresponding **vitamin D**.sub.3 analogues. For example, in parent co-pending application, Ser. No. 08/265,438, the disclosure of which is

incorporated herein by reference, the. . .

SUMM The hypocalcemic **vitamin D** compounds of the present invention are useful as active compounds in pharmaceutical compositions having reduced side effects and low toxicity as compared with the known analogues of active forms of **vitamin D**.sub.3.

SUMM . . . conventional methods of pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans. For example, the hypocalcemic **vitamin D** compounds can be employed in admixtures with conventional excipients, e.g., pharmaceutically acceptable carrier substances suitable for enteral (e.g., oral), parenteral. . .

SUMM . . . wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or one or more other active compounds, for example, **vitamin D**.sub.3 and its 1.alpha.-hydroxylated metabolites, conjugated estrogens or their equivalents, anti-estrogens, calcitonin, biphosphonates, calcium supplements, cobalamin, pertussis toxin and boron.

SUMM . . . 0.01 .mu.g to about 50 .mu.g per gram of composition. For treatment of skin cancers, the dosage of the hypocalcemic **vitamin D** compound in a locally applied composition generally is about 0.01 .mu.g to 100 .mu.g per gram composition.

SUMM Further, included within the scope of the present invention is the co-administration of a hypocalcemic **vitamin D** compound with a anticancer agent, e.g., a cytotoxic agent, Such agents suitably include antimetabolites (e.g., 5-fluoro-uracil, methotrexate, fludarabine), antimicrotubule agents. . . daunomycin), topoisomerase inhibitors (e.g., etoposide, camptothecins) or any other antineoplastic agents. (estramustine phosphate, prednimustine). It is anticipated that the hypocalcemic **vitamin D** compounds used in combination with various anticancer drugs can give rise to a significantly enhanced cytotoxic effect on cancerous cells,. . .

SUMM . . . other at a later time, typically within a week. An example of a suitable co-administration regimen is where a hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of a cytotoxic agent.

SUMM . . . of hormones or other agents, e.g., estrogens, which are known to ameliorate bone diseases or disorders. As noted above, prostate **cancer** often metastasizes to bone, causing bone loss and associated pain. Such bone agents may include conjugated estrogens or their equivalents,. . .

DETD The affinity of 1.alpha.,24-(OH).sub.2D.sub.2 for the mammalian **vitamin D** receptor (VDR) was assessed using a commercially available kit of bovine thymus VDR and standard 1,25-(OH).sub.2D.sub.3 solutions from Incstar (Stillwater,. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD The VDR affinity binding of 1.alpha.,24-(OH).sub.2D.sub.4 was investigated. The 1.alpha.,24-(OH).sub.2D.sub.4 was incubated with **vitamin D** receptor and radiolabeled tracer 1.alpha.,25-(OH).sub.2D.sub.3. After incubation, the amount of radioactivity bound to the receptor was determined and compared with. . .

DETD These results show that 1.alpha.,24-(OH).sub.2D.sub.4 binds slightly less tightly to the **vitamin D** receptor than does 1.alpha.,25-(OH).sub.2D.sub.3. Such data mean that 1.alpha.,24-(OH).sub.2D.sub.4 has high affinity for the VDR and significant biological activity, similar. . .

DETD . . . results are surprising and unexpected in view of the prior art. They are contrary to the normative wisdom in the **vitamin D** art regarding the very low degree of biological activity of **vitamin D.sub.4** compounds.

DETD VDR binding of **vitamin D** compounds by prostate cells

is demonstrated using the techniques of Skowronski et al., 136 Endocrinology (1995) 20-26, which is incorporated. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.** **4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,24-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. .

.

DETD The procedure of Example 3 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the specific binding is determined. The results demonstrate that 1.alpha.,25-(OH).sub.2D.sub.4 has strong affinity for prostate VDR, indicating. .

.

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.** **4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD Using the plasmids p(CT4).sup.4TKGH, a **vitamin D** receptor (VDR)-expressing plasmid, and pSG5-hVDR1/3, a plasmid containing a Growth Hormone (GH) gene, under the control of a **vitamin D**-responsive element (VDRE), experiments were conducted to explore the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to induce **vitamin D**-dependent growth hormone acting as a reporter gene compared to that of 1.alpha.,25-(OH).sub.2D.sub.3. Cells in culture were transfected with these two plasmids. One plasmid contained the gene for Growth Hormone (GH) under the control of the **vitamin D** responsive element (VDRE) and the other plasmid contained the structural gene for the **vitamin D** receptor (VDR). These transfected cultures were incubated with 1.alpha.,24-(OH).sub.2D.sub.4 or 1.alpha.,25-(OH).sub.2D.sub.3, and the production of growth hormone was measured. Table. . .

DETD

TABLE 2

Induction of Growth Hormone by **Vitamin D** Compounds

Compound Used	Concentration (M)	Growth Hormone Induction (ng/ml)
1,25-(OH).sub.2D.sub.3	1 .times. 10.sup.-10	39
1,25-(OH).sub.2D.sub.3	5 .times. 10.sup.-10	248
1,24-(OH).sub.2D.sub.4	5 .times. 10.sup.-10	165
1,24-(OH).sub.2D.sub.4.	. .	

DETD These data show that the ability of 1.alpha.,24-(OH).sub.2D.sub.4 to stimulate **vitamin D**-dependent growth hormone is nearly equivalent to that of 1.alpha.,25-(OH).sub.2D.sub.3. Such results are truly surprising and would not have been expected. . .

DETD 1.alpha.,24(S)-dihydroxyvitamin D.sub.2 and 1.alpha.,24(R)-dihydroxy-**vitamin D.sub.2** [1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2]

DETD . . . was conducted to compare the biological activity in vitro of chemically synthesized 1.alpha.,24(S)-(OH).sub.2D.sub.2 and 1.alpha.,24(R)-(OH).sub.2D.sub.2, with 1.alpha.,25-(OH).sub.2D.sub.3 and 25-OH-D.sub.3. The **vitamin D**-dependent transcriptional activation model system was used in which plasmids pSG5-hVDR1/3 and p(CT4).sup.4TKGH were co-transfected into Green monkey kidney, COS-1 cells.

DETD Transfected cells were incubated with **vitamin D** metabolites and growth hormone production was measured. As shown in Table 3, both 1.alpha.,24(S)-(OH).sub.2D.sub.2 and its epimer, 1.alpha.,24(R)-(OH).sub.2D.sub.2, had significantly. . .

DETD

TABLE 3

Vitamin D-Inducible Growth Hormone Production
In Transfected COS-1 Cells
Vitamin DCInducible Growth
Hormone Production
Net
Molar Total GH vitamin DCinducible
Concentra- Production* GH-production
Inducer. . .
DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. Medium containing test analogue or vehicle is replaced every three. . .

DETD 1.alpha.,24-dihydroxy **vitamin D.sub.4** [1.alpha.,24-(OH).sub.2D.sub.4]

DETD The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,24-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD The procedure of Example 8 is repeated using the active **vitamin D** analogue 1.alpha.,25-(OH).sub.2D.sub.4, and the cell number is determined. Cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly fewer cells than the control cultures.

DETD . . . the cells have attached and stabilized, about 2-3 days, the medium is replenished with medium containing vehicle or the active **vitamin D** analogue, 1.alpha.,24-(OH).sub.2D.sub.2, at concentrations from 10.sup.-11 M to 10.sup.-7 M. After 6-7 days, the medium is removed and stored at. . .

DETD The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,24-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,24-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD The procedure of Example 12 is repeated except the active **vitamin D** analogue is 1.alpha.,25-(OH).sub.2D.sub.4. The PSA is measured and cultures incubated with 1.alpha.,25-(OH).sub.2D.sub.4 have significantly more PSA than control cultures when. . .

DETD Patients with malignancy-associated hypercalcemia participate in an open-label study of a hypocalcemic **vitamin D** compound in accordance with the present invention. Patients are restricted to daily calcium intake of about 400-500 mg. Each patient. .

CLM What is claimed is:

. . . of treating hypercalcemia associated with malignant or neoplastic cells, comprising treating the cells with an effective amount of a hypocalcemic **vitamin D** compound having a hydrocarbon moiety at the C.sub.24 position.

2. The method of claim 1, wherein the cells are cancers of the breast, colon, **lung**, neck and head, pancreas, endometrium, bladder, cervix, testes, ovaries, squamous cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma,. . .

3. The method of claim 1, wherein the hypocalcemic **vitamin D** is a compound represented by formula (I) ##STR4## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon bond, thus. .

4. The method of claim 1, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxvitamin D compound is represented by formula (III) ##STR5## wherein A.sup.1 and A.sup.2 each are hydrogen or a. . .

6. A method in accordance with claim 1, wherein a dosing regimen for the

hypocalcemic **vitamin D** compound is a daily regimen or an episodic regimen.

8. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is administered daily at a dose of about 10 to 100 .mu.g/day.

9. A method in accordance with claim 6, wherein the hypocalcemic **vitamin D** compound is orally, intravenously or regionally delivered to a **cancer** site.

10. A method in accordance with claim 9, wherein the hypocalcemic **vitamin D** compound is administered orally.

11. A method in accordance with claim 1, wherein the hypocalcemic **vitamin D** compound is co-administered with a cytotoxic agent.

22. A method of treating a human to alleviate hypercalcemia associated with breast **cancer**, colon **cancer**, prostate **cancer**, testicular **cancer**, pancreatic **cancer**, endometrial **cancer**, small cell and non-small cell **cancer** of the **lung** (including squamous, adneocarcinoma and large cell types), squamous cell of the head and neck, bladder, ovarian and cervical cancers, myeloid. . . melanoma, retinoblastoma or sarcomas of the soft tissue and bone, comprising administering to the human therapeutic amount of a hypocalcemic **vitamin D** compound.

23. A method of claim 22, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula (III) ##STR6## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon. . .

. . A method of treating a human to alleviate hypercalcemia associated with malignant cells, comprising administering to the patient a hypocalcemic **vitamin D** compound, and a cytotoxic agent.

27. A method in accordance with claim 26, wherein the hypocalcemic **vitamin D** compound is administered from 0.5 to 7 days prior to administration of the cytotoxic agent.

28. A method in accordance with claim 26, wherein the hypocalcemic **vitamin D** compound is administered 2 to 4 days prior to administration of the cytotoxic agent.

29. A method of claim 26, wherein said hypocalcemic **vitamin D** is a 1.alpha.-hydroxyvitamin D compound represented by formula (III) ##STR7## wherein A.sup.1 and A.sup.2 each are hydrogen or a carbon-carbon. . .

. . serum parathyroid hormone related protein in a human patient by administering to the human an effective amount of a hypocalcemic **vitamin D** compound.